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AUSTRALASIAN ANNALS OF MEDICINE

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DE MOTU CORDIS : CELLULAR PHYSICS AND CHEMISTRY[1]

W. V. MACFARLANE[2]

From the Sir William Macgregor School of Physiology, University of Queensland

SUMMARY

William Harvey regarded himself as an anatomist although he provided the first physiological analysis of the circulation. In this he used the physical concepts which Galileo taught while Harvey was at Padova, as well as the experimental method and inductive-deductive sequence of logic which the New Philosophy recommended as an approach to understanding. His hypothesis was supported shortly after his death by Malpighi's discovery of the capillaries in 1661. The circulation of the blood was expounded in medical schools in physical terms. It was not until late in the eighteenth century that quantitative chemistry began, and at that time the first suggestion that the heart might require energy for its pumping action was made by Felice Fontana of Bologna. During the nineteenth century physical analysis of heart action in terms of pressures, velocities, sound and electrical activity went forward. The metabolic background was, however, slow to develop, and only in the nineteen-thirties was it clear that oxygen and lactate were the energy sources of heart action.

In the last ten years some attempt at synthesis of the physical and the chemical background of heart action has been made. Single cells studied chemically and physically yield a picture of the heart as a whole. Using intracellular electrodes and metabolic blocking agents it is found that the duration of the refractory period is lessened in the absence of oxygen or in the presence of agents decoupling oxidative phosphorylation. The rest pause before any particular action potential of the cell determines the length of that action potential and therefore of the refractory period. High energy phosphate is probably necessary for the maintenance of refractoriness.

Agents (dinitrophenol, digitalis glycosides, sodium azide or hypoxia) which reduce the duration of the action potential also slow the heart, when innervation of the heart has been interrupted. It is possible that the initiation of heart beat is an active process of ion transfer employing the same energy sources as those producing the refractory period.

The physical manifestations of heart action studied by Harvey thus meet the chemical sources of energy at the cellular level where analysis of the motion of the heart must ultimately take place.

HARVEY regarded himself as an anatomist, and since in addition he was a friend and pupil of Fabricius ab Aquapendente, he was identified with anatomy by his contemporaries. Yet Harvey realized that he used the new experimental methods and that his were functional studies. He was influenced strongly by the sixteenth and seventeenth century rediscovery of an experimental approach to natural knowledge. Francis Bacon was one of his patients, but Harvey had seen the practice of contemporary ideas on method before Bacon published his *Novum Organum* as an exposition of the new science. The Venice merchants had encouraged a new freedom in the University of Padova, where Harvey studied from 1598, and where Galileo Galilei had come to work and teach in 1592; so the physician from Cambridge was exposed to an atmosphere charged with all that was modern in the science of his day. Harvey followed the method of observation, induction to hypotheses and quantitative experimental test in his search for understanding of the action of the heart.

The method of experimental science had been described clearly by Robert Grosseteste, the first Chancellor of Oxford, in 1209 (Crombie, 1953) in his commentaries on Aristotle. His fellow Franciscan and pupil, Roger Bacon, used the experimental method on occasion to resolve physical problems. More than four hundred years elapsed between Grosseteste's exposition and Harvey's publication of *Exercitatio Anatomica de Motu Cordis et Sanguinis in Animalibus*. Between Grosseteste and Harvey lay the Hundred Years' War and the Black Death, so that the new science underwent 400 years of gestation before it began its period

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of rapid growth. The growth rate increased with Harvey's successful use of experiment, and this stimulated more biological trial and error in the seventeenth century.

The approach made by Harvey was initially descriptive, but his experiments were physical. He felt the muscle of the heart harden in his hand. He measured the volume of flow along a vein. He did not describe a pump in so many words, but in 1616 he thought the heart acted like " the two clacks of a water bellows to rayse water ". Servetus, Columbus and Cesalpinus had groped after an interpretation of the heart and were confused by the idea that its main function was the sucking of blood during diastole. Harvey realized clearly that " the motion is just the same as that of muscles " ; " the heart while contracting empties " (contrary to the commonly held opinion) ; and " the [frog] heart becomes pale when it squeezes the blood out during contraction, but when quiet in relaxation the deep blood-red colour returns as the ventricle fills again with blood " (Leake, 1941).

The obvious deductions from Harvey's theory of circulation were sought and tested. In 1661 Marcello Malpighi (who taught the new doctrines of circulation at Pisa) saw that venous blood flowed from the right side of the heart through capillaries in the lungs of frogs to the left side of the heart. Lower, at Oxford, within ten years of Harvey's death, was able to show that air changed dark blood to clear red blood as it passed through the lungs. This was the first step in explaining the function of respiratory movements. Harvey knew that blood must pass through the lungs, but he could offer no other reason for this than that of cooling the blood, as Galen had suggested. Pepys at the Royal Society recorded the opinion of George Ent (a close friend of Harvey) on respiration that " it is not concluded among physicians . . . for what use it is ". Hooke in 1667 and Mayow in 1668 showed by experiment that the pulmonary circulation was concerned to change the colour of venous blood and add nitro-aerial spirit to it from the air.

Thus slowly the theory of circulation was tested and extended as its influence spread.

Although the physical methods Harvey used were simple, physicists like Boyle appreciated the overall lucidity of his demonstrations. Physics continued to grow in stature and scope throughout the seventeenth century, but it was not until the end of the the eighteenth century that chemistry became a quantitative science. Shortly before the French revolution, when Lavoisier was able to isolate and describe the properties of oxygen, the Abbate Felice Fontana of Bologna speculated on the action of the heart. He thought that diastole was a period of rest for the heart during which restitution of energy resources took place before the next beat (Hoff, 1942). At the end of systole he could not induce another beat by pricking the heart, though this was possible later in diastole. The combination of physical concepts with biochemical mechanisms that he suggested is still not complete, but the convergence of the two approaches may be examined further.

During the nineteenth century physical records of contraction, pressure and electrical activity were made by Schiff, Kronecker and Bowditch amongst others. At the Collège de France in Paris, Marey produced extrasystoles by electrical stimulation, recorded them graphically and demonstrated the refractory period (which he named). His records published in 1876 show that a premature mechanical beat was shorter in duration and lower in total amplitude than a normal beat. Marey was more concerned with the compensatory pause and the refractory period than with the rather diminished extrasystole. Trendelenburg in 1903 emphasized the short course and low amplitude of an early extrasystole. It was Adrian, however, using a Lucas capillary electrometer to measure the injury potential of frog hearts in 1921, who made it clear that the unit electrical activity of the heart was a prolonged depolarization corresponding in time with the refractory period (Figure I*a*). An extrasystole was shorter in duration than the normal electrical event. Interpretation of Adrian's findings became clearer after the use of intracellular electrodes began in Chicago in 1946. Adrian had recorded, in essence, the single fibre action potential. From 1946 onwards, it became possible to record and analyse cellular electrical events in the heart. These unit potentials form the basis of the electrocardiogram.

The biochemical attack had from other origins been converging on the cellular physics of the heart. Oxygen and lactate (Himwich, Koskoff and Nahum, 1930 ; McGinty, 1931) were found to be the chief energy-yielding system of the heart. Lactate provides energy for contraction as well as for the electrical triggering of heart action. The essential energy supply of the heart comes from the oxidation of lactate, and this energy is stored as high-energy phosphate—as creatine phosphate or adenosine triphosphate. These energy reserves built up by the action of mitochondrial enzymes (Green, 1951) are available both for contraction and for electrical recovery processes.

An attempt may now be made to join some of the biochemical machinery of the cell with

intracellular records of electrical events of the heart. Intracellular electrodes and metabolic inhibitors provide one possible linkage between the physical and chemical aspects of cell function.

Glass electrodes with 0·5μ tips were devised by Graham and Gerard (1946). They may be thrust into a single fibre of the beating heart for

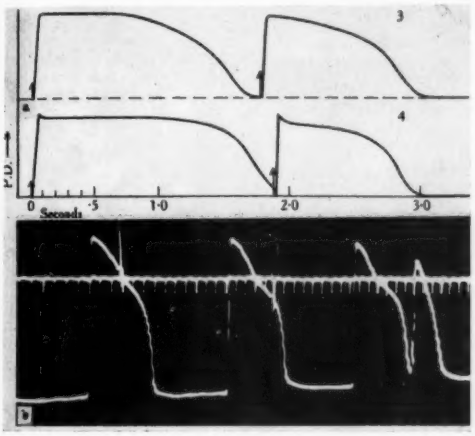

FIGURE I

(*a*) Monopolar injury potentials of frog heart from Adrian (1921). The refractory period (at 14° C.) lasted nearly two seconds, and the extrasystole had a duration of only half that of the normal action potential.

(*b*) Frog heart, intracellular recording of ventricle at 22° C. The action potentials show rapid depolarization, overshoot of the zero potential baseline, and a slow recovery process. Test shocks applied during the plateau of depolarization in the first two potentials produced no extrasystole. In the third, as the potential approached the resting level, an extrasystole was induced. It has less overshoot and a shorter plateau than the normal action potential. Time: 100 milliseconds

half an hour if necessary, and they cause little damage to the cell. Records have been obtained from the hearts of amphibia, chick embryos, dogs, cats, guinea-pigs, goats, mice, rats and man. Fibres of sinus, auricle, ventricle, Purkinje systems or *conus arteriosus* (Woodbury and Brady, 1956; Brooks, Hoffman, Suckling and Orias, 1955) have been investigated. In our laboratory, most investigation has been undertaken with amphibian hearts in the tradition of Harvey ("the movement is more easily seen in the hearts of cold-blooded animals as toads, snakes, frogs, snails . . . ").

Some caution is necessary in extrapolating from one species to another, but there seem to be only minor differences in cell behaviour between mammals, while the hearts of amphibia function electrically very like those of mammals.

As a microelectrode pierces the membrane of a heart fibre, a potential difference of −85 to −95 millivolts is instantly recorded between the microelectrode and an indifferent electrode in the tissue fluid. When the cell becomes active, the resting membrane potential is discharged through zero and overswings by 40 to 50 millivolts in a positive direction. Depolarization takes about 50 microseconds and, according to Draper and Weidmann (1951), it is the result of a rapid increase in inward diffusion of sodium ions. This is followed by the beginning of an outward diffusion of potassium or by slowing of sodium penetration. In most heart tissues there is a pause at this stage, during which the potential maintains a plateau of depolarization near zero for about 0·5 second. The potential then falls to the resting membrane potential as it repolarizes. This is followed by an electrical pause lasting approximately 0·5 second (Figure I*b*).

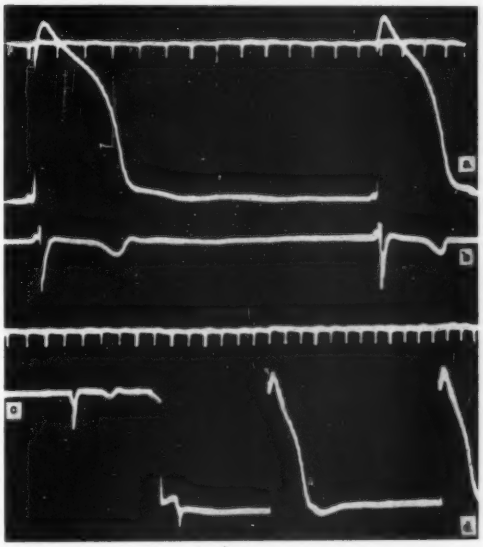

FIGURE II

Comparison of extracellular and intracellular potentials. (*a*) Intracellular record of frog ventricle fibres. (*b*) Extracellular record immediately after the electrode was withdrawn from the fibre of (*a*). (*c*) Extracellular *QRS* and *T* waves. The electrode is advanced and penetrates the cell. (*d*) Intracellular action potentials corresponding with (*c*). The *QRS* electrogram corresponds with depolarization and the *T* with the end of repolarization of single fibre action potentials. Time: 100 milliseconds

Hoffman and Suckling (1953) correlated the electrogram of the heart of a dog with intracellular ventricular potentials. In Figure II*a* and *b*, the surface electrogram of a frog ventricle

is similarly related to the intracellular potential. Figure II*c* shows a volume conductor record with a *QRS* and *T* complex, taken from the surface of a ventricular fibre without penetration by the microelectrode. The electrode then pierces the membrane (II*d*), and the resting potential is recorded. A sinus-driven discharge of the cell is then recorded as the intracellular potential rises steeply, overshoots the zero baseline and slowly returns to the resting voltage. The main variations of this pattern may now be considered.

Different Heart Cells

Atrial Fibres.—In amphibia and mammals the atrial fibres repolarize faster than those of the ventricle. This repolarization is speeded up when acetylcholine acts on the atrium or when the vagus is stimulated (Hoffman and Suckling, 1953). Hutter and Trautwein (1955) at Johns Hopkins Hospital observed a further specialization of auricular activity. This was the increase of polarization (greater resting membrane potential) of the sinus node during the action of the vagus. Increase in resting potential was found to depress excitability at the sinus and to slow the repetition rate of sinus discharge.

Purkinje Fibres.—The goat heart studied by Draper and Mya Tu (1955) has Purkinje fibres with a lower resting potential than those of the atrium or ventricle but with a longer plateau than either atrium or ventricle.

Ventricle.—Mammalian and amphibian ventricular fibres depolarize rapidly. The repolarization process is slower than in the atrium. All fibres in the ventricle do not, however, have the same time-course of recovery. The plateau near zero potential may be maintained longer on one side of the heart than the other and recovery is a little faster at the apex than at the base of the frog heart (unpublished observations). Acetylcholine and the vagus nerve have little effect on ventricular repolarization. On the other hand adrenaline delays repolarization and maintains the plateau of depolarization in all types of cell.

Changes of Repolarization Time

The *T* wave corresponds with the return of the action potential to resting level (Figure II*a* and *b*). Repolarization time (or the *Q-T* interval) may be modified in several ways:

1. The rate of beating of the dog heart was shown by Hoffman and Suckling (1954) to determine the slope and duration of the repolarization phase. This applies to both atrium and ventricle. An auricle beating at 60 per minute repolarizes in about 400 milliseconds, whereas at 220 per minute it is repolarized in about 100 milliseconds without a plateau. Similarly, ventricle fibres may be driven at rates up to 240 per minute, so that repolarization occupies less than 200 milliseconds. We have shown on the excised amphibian heart (Macfarlane, 1956) that the length of the

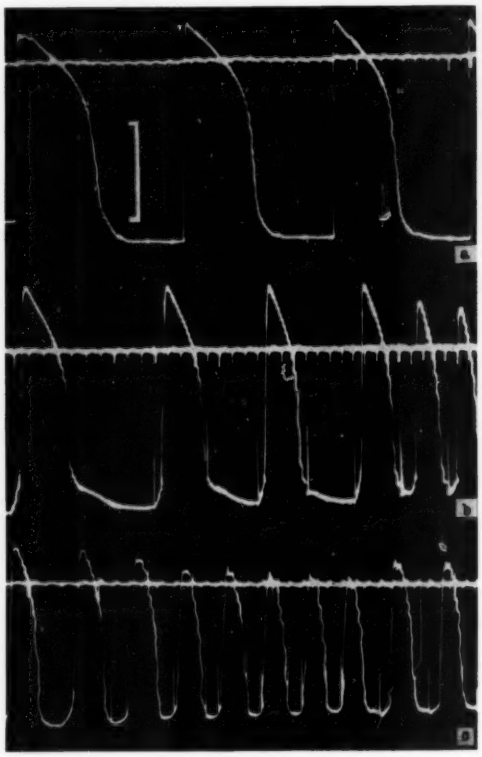

FIGURE III

Frog ventricle fibres at 24° C. (*a*) Normal heart beat, with 0·5 second action potentials followed by 0·5 second rest pause. (*b*) Action potentials after a period of fast activity induced by electrical stimulation. The action potential is reduced to 0·35 second duration in spite of 0·65 second rest between beats. There is partial fatigue. The heart is then driven more rapidly. (*c*) As the heart rate increases there are three changes in action potential: (i) The plateau becomes shorter proportionally to the reduced rest pause preceding any action potential. (ii) There is reduced resting potential and overshoot. (iii) The rate of depolarization is slowed

preceding rest phase determines the duration of the subsequent plateau (Figure III). If, therefore, the ventricle is activated through the Purkinje system before the electrical rest period is half finished, there is a proportional reduction in the plateau of depolarization; so recovery time is half that of the preceding

beat, and the refractory period is correspondingly shorter. At heart rates slower than 40 per minute, the diastolic rest phase does not remain proportional to the repolarization time. But at rates above 40 per minute, the regression of resting phase on repolarization is linear (Figure IV). The plateau, therefore, is short unless preceded by an adequate electrical pause at resting potential. A similar type of behaviour occurs with extrasystoles. In Figure I*b* are shown records of stimuli applied to the heart during the repolarization phase. The refractory period lasts until repolarization is nearly complete; so no extrasystole is induced until the resting potential is approached. When

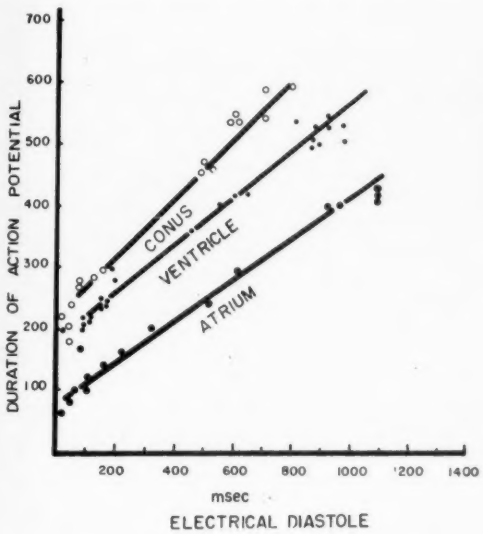

FIGURE IV

extrasystoles appear they have a repolarization time of 310 milliseconds compared with the preceding action potential of 630 milliseconds at 22° C. This shortening of the *Q-T* interval of a premature beat was observed by Trendelenburg (1903), and by Adrian (1921) using injury potentials in the frog, while Hoffman and Suckling (1954) in dog hearts found similar behaviour. The records made by Samojloff (1910) of premature beats show not only shorter electrical activity, but also less mechanical force than a normal beat. Very rapid stimulation leads to action potentials lasting only 50 to 100 microseconds. These low voltage 10 to 20 per second events in cells (Figure V) lead to fibrillation. It seems probable that most forms of fibrillation involve shortening of the refractory period. Agents shortening refractory period (such as digitalis, hypoxia or acetylcholine on the atrium) lead also to tachycardia or fibrillation (*cf.* Burn and Goodford, 1957).

2. Alteration of the electrolyte constituents of the extracellular medium changes the time of repolarization. In the isolated amphibian heart we have found that reduction of extracellular potassium causes a slight lenthening of repolarization time (20% increase), whereas increasing potassium to twice or thrice the normal concentration reduces repolarization time (up to 50%). Low and high calcium concentrations in the extracellular medium act in the same direction as potassium. This is particularly so in the atrium (Hoffman and Suckling, 1953).

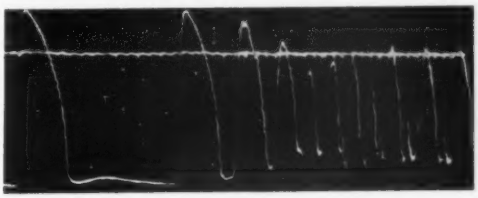

FIGURE V

Ventricle driven at 10 per second. There are irregular action potentials and fast, local responses as in fibrillation, with 100 milliseconds or less for repolarization

3. Raising the temperature of heart cells speeds up repolarization and shortens the refractory period, whereas cooling slows recovery. It is possible to drive an amphibian heart into tetanus when the temperature is raised, because the refractory period is shortened (Whitehorn, 1953). Whitehorn found that digitoxin (which shortens the plateau) behaves like raised temperature and allows tetanus to develop, but this is prevented by adrenaline (which lengthens the plateau in excised hearts).

4. Autonomic activity results in changes both of rate and of repolarization. Acetylcholine accelerates the rate of single fibre repolarization of any fibres receiving vagus innervation. In mammals the ventricle is not, therefore, affected by acetylcholine. Acierno *et alii* (1952) have counteracted this effect of acetylcholine by using adenosine triphosphate (ATP). The shortening of atrial repolarization by acetylcholine is likely, therefore, to be due to inhibition of an electrolyte "pump" using ATP energy.

5. Metabolic inhibitors and drugs. We have observed that the duration of the action potential is prolonged by adrenaline and veratrine (*cf.* Cohen and Meilman, 1955).

Shortening of the plateau of depolarization in atria occurs under the influence of digitalis glucosides (Woodbury and Hecht, 1952) and acetylcholine. We have found (Macfarlane and Meares, 1955; Marshall, 1955) that metabolic inhibitors (2 : 4 dinitrophenol (DNP), neutral red, sodium azide) rapidly reduce the time of the repolarization phase in atrium, ventricle and truncus. Loomis and Lipmann (1949) have provided evidence that the main action of these substances is decoupling of oxidative phosphorylation or reduction of available ATP. Before any significant loss of resting potential occurs, the action potential shortens to one-sixth of its normal duration. The plateau is short or absent, and it is independent of the heart rate (Figure VI). A long rest pause does not lengthen the action potential after DNP treatment. All heart fibres in amphibia are not equally sensitive to the action of DNP. Fibres in the *truncus arteriosus* of the frog resist 0·5 millimolar DNP, although the repolarization time of ventricle and atrial cells is reduced to one-third at that concentration (unpublished observation). Although other actions are possible, the most likely explanation for the effect of DNP is that it reduces the phosphate energy available from creatine phosphate or ATP by decoupling of oxidative phosphorylation (Loomis and Lipmann, 1948). In the absence of these energy sources repolarization takes place rapidly, instead of work being done through $\sim PO_4$ to maintain the fibres at a plateau near zero potential, probably by active ion transport.

No intracellular effect of digitalis glucosides appears to have been recorded other than the potentiation of decoupling of oxidative phosphorylation, observed by Grisolia (1955) in liver mitochondria. Shortening of repolarization time by digitoxin parallels the action of DNP, and both probably interrupt active ion transport. In skeletal muscle, potassium diffuses rapidly out of the active cell to restore the resting potential within two milliseconds (Nastuk and Hodgkin, 1950). This is delayed in cardiac muscle by the plateau, which probably represents a period during which potassium influx or chloride efflux is brought about by active transport.

Weidmann (1951) measured the impedance of Purkinje fibres and found that there was a low conductance during the plateau. Lepeschkin (1955) has suggested that the plateau results from changes in the outflux of potassium. The net potassium transfer would be zero; so conductance would be low. It seems more likely, however, that a state of ionic equilibrium is maintained in heart cells by inward pumping of potassium to counteract the normal outward diffusion of that ion down the electrochemical gradient from the cell. Evidence has been provided by Hodgkin and Keynes (1954) and Coombs, Eccles and Fatt (1955) for active inward transport of potassium by nerve. Shanes and Berman (1956) report active potassium influx in nerve which is blocked by hypoxia and metabolic inhibitors, and Börn and Bülbring (1955) find active influx of K^+ in smooth muscle.

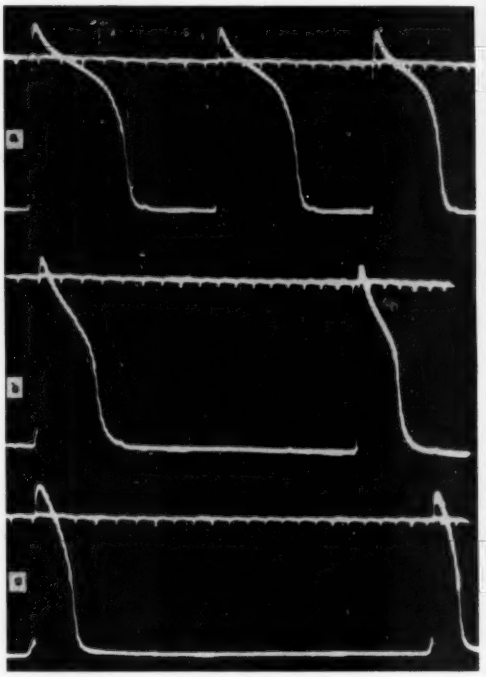

FIGURE VI

Frog ventricle fibres at 24° C. (*a*) Normal beating of excised heart in Ringer solution. (*b*) After five minutes in 0·2 millimolar 2 : 4 DNP. The duration of the action potential was reduced from 0·54 to 0·33 second and the sinus discharge rate slowed. (*c*) After twenty minutes in 0·2 millimolar DNP, the action potential was reduced to 0·2 second and the heart rate was further slowed. Resting potential fell 20 millivolts. In the presence of DNP a long rest pause does not lead to a long plateau as it would in a normal heart

This is suggestive of a similar process in heart muscle, where K^+ influx would prevent repolarization during the plateau. Chloride extrusion could bring about a plateau in a comparable fashion and maintain refractoriness. This metabolic hypothesis of the refractory period is supported by the association of a short diastole with a short repolarization time, and by the effects of decoupling oxidative phos-

phorylation in shortening the *Q-T* segment. Both would decrease the replacement of ATP or creatine phosphate.

HEART RHYTHMICITY

The concept of the refractory period as a consequence of active ion transport may be extended to the intermittency of sinus node discharge or of any other cardiac pacemaking cells. If active depolarization occurred in the nodal cells, and the amount of energy available for ion transport were limited, there would be rhythmic discharge independently of nerves. Transmitter substances from cardiac nerves only alter the intrinsic rate of heart beat.

The evidence in favour of this hypothesis comes from several sources. Weidmann (1951) has shown that there is a prepotential (depolarization) at the sinus node, prior to the initiation of an action potential. Acetylcholine slows the heart and increases the resting potential—that is, it hyperpolarizes the sinus cells and reduces the chance of their discharging (Hutter and Trautwein, 1955) by slowing the rate of depolarization. While it slows the heart, acetylcholine also shortens the refractory period of the atrium but not of the ventricle. Probably acetylcholine allows diffusion of K^+ to its full equilibrium potential in the specialized atrial cells on which the vagus ends (West, Falk and Cervoni, 1956).

A similar slowing, with reduced refractory period in atrium and ventricle, is produced by DNP (Figure VI) and by digitalis glucosides. Both these substances reduce oxidative phosphorylation, and it is probable that the lack of action potential plateau produced by DNP results from this limitation of energy for ion transport. If initiation of a sinus beat required active depolarization, there would be reduction of this process when ATP supply was reduced, and slow depolarization would slow the heart. If shortening of the action potential represents reduced energy for active ion transport, then slowing of the heart by metabolic blockade is evidence for active transport in prepotential depolarization.

Adrenaline on the other hand prolongs the refractory period (at any given heart rate) and increases the force of contraction. It seems likely that it increases active ion transport. The chronotrophic effect in speeding up the sinus discharge rate is explicable as increased rate of depolarization in the prepotential stage, leading to frequent discharge. This is shown in the records of Hutter and Trautwein (1955), where adrenaline was found to produce frequent, fast-rising prepotentials in sinus node fibres. As soon as an action potential finished, energy for a new depolarization would be available rapidly to initiate a new action potential.

No direct evidence of the effects of inhibitors of phosphorylation on prepotential depolarization in the sinus node has yet been obtained; but if the hypothesis should be correct, there would be slow rates of rise of depolarization in the presence of digitalis, DNP, azide and similar substances that reduce energy supplies.

There are testable deductions from these hypotheses, and in the fashion of Grosseteste or Harvey the process of experimental verification or falsification will go on. Isotopic K or Cl should reveal the direction of ion flow when the plateau is shortened, as by acetylcholine or DNP. The available ATP should be measurably reduced in fast-driven hearts. Oxygen, lactate or intracellular injection of ATP should increase the refractory period, and increase the heart rate of isolated hearts. There is yet no clear answer on the different mechanisms of absolute and relative refractory periods, nor on the supernormal phase during diastole. Each answer frames itself into a new question, and each new answer will be incomplete. Harvey bequeathed his estate to the Royal College of Physicians in 1658, and he directed that they use it "to search out and study the secrets of nature". He must have known there were no quick answers.

CONCLUSIONS

William Harvey knew the movement of the heart as a palpable rising, hardening and contraction of muscle. As chemical processes have been identified in the physical events of excitation, conduction, contraction and refractoriness, physical and chemical approaches have converged upon the cell. In spite of any anatomical description of a cardiac syncytium, heart fibres function as cells.

With intracellular electrodes, some link may be detected between refractory period and energy supplies. It is postulated that the refractory period results from an active process of ion transport which maintains the cell potential near zero, so that heart fibres are inexcitable. Evidence for this is derived from the following:

1. The short duration of the action potential of an early extrasystole—probably because of insufficient rest to accumulate energy for the next plateau.

2. The dependence of the duration of each action potential upon the length of diastolic rest preceding it; as the heart rate increases there is automatic reduction of refractory period, since (it is postulated) high energy phosphates are not sufficiently available for a longer plateau.

3. The reduction of available high energy phosphate by dinitrophenol, azide and hypoxia, which leads to shortening of the action potential, regardless of the rest-pause between beats. The hypothesis that most readily accommodates these observations is that an active inward transport of ions occurs to maintain the fibres of the heart in a refractory state near zero potential. When this ceases, diffusion outwards of potassium restores the fibres to excitability at a resting potential near −85 millivolts.

4. This concept may be extended to account for the intrinsic rhythm of the heart as an active depolarization. The better the energy supply for depolarization, the faster the beat; and when metabolic inhibition occurs, the beat is slowed.

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HISTOCHEMICAL DIFFERENCES BETWEEN REGENERATING AND NEOPLASTIC RAT LIVER[1]

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SUMMARY

Fluorescent sera obtained by conjugating fluorescein isocyanate with the globulin fraction of serum obtained from rabbits previously injected with homogenates of normal rat liver have an affinity for rat liver and regenerating rat liver but not for rat hepatoma. There is a close correlation between these staining reactions and alterations occurring in cellular proteins during carcinogenesis; these may be demonstrated electrophoretically. The phenomenon is an expression of an essential difference between neoplastic and normal tissue and is not an indication merely of cellular activity.

ONE of the most significant advances in cancer research of this century has been the series of observations that, in the case of certain chemically induced tumours, neoplastic cells differ from those of the originating tissue in that they lack certain protein complexes present in the normal cells. The matter is an involved and complicated one, but the main features may be mentioned briefly. In rat liver the carcinogen has been demonstrated (initially because it is coloured) to become attached to a particular protein component of the cell protoplasm. This protein(s) has been segregated by high speed centrifugation and by electrophoresis; its general characters have gradually been elicited.

The protein complexes have been shown to be absent from the cells of the developed tumour and diminished in some cells in the pre-neoplastic stage. Various hypotheses have been put forward of the nature both of this change and of the particular proteins concerned, and the question is immediately posed whether the phenomenon is one of simple tissue growth or is peculiar to the neoplastic process.

One method of demonstrating the difference between the neoplastic and normal cells was by the production of antibodies to the normal cells by injecting normal tissue suspensions into rabbits. The antibodies so produced—γ-globulins—may be visualized by conjugating them with fluorescing dyes. The presence or absence of the proteins may be determined by observing the areas which fluoresce when the fluorescing rabbit serum is used as a stain for the sections. This technique has been developed and adequately described by Coons and Kaplan (1950).

This immunological approach to the problem has led to various assumptions. Thus Weiler (1952, 1956*a*, *b* and *c*) reported that malignant cells of rat hepatomata induced by feeding 4-dimethylaminoazobenzene and stilbœstrol-induced renal tumours in the hamster lacked the " organ-specific antigens " of the parent cells. He presented serological and histoserological evidence to support his claims.

He found that, following the injection of cellular particulates prepared from rat liver into rabbits, an antiserum could be produced which reacted in complement-fixation tests with rat liver particulates in higher titre than with rat kidney particulates. This antiserum, after repeated absorptions with rat kidney particles (to remove antibodies which are not organ-specific), was found to give no reaction in complement-fixation test with rat kidney particles, but still reacted in high titre with rat liver particles; in other words, the antiserum is organ-specific. Following conjugation with fluorescein isocyanate by the method of Coons and Kaplan (1950) and subsequent absorption with rat kidney particles, a fluorescent " organ-specific " antiserum could be obtained. Weiler (1956*a* and *c*) found that by using such a fluorescent organ-specific antiserum as a histo-serological stain, it was possible to stain normal but not malignant rat-liver and hamster-kidney tissues.

Hughes *et alii* (1957) could not produce an organ-specific antiserum using Weiler's methods, but found that fluorescent normal rabbit globulin gave differential staining similar to that obtained by Weiler (1956*a*). This

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phenomenon is an important one, but appears not to be as specific as the hypothesis would suggest.

To decide if this gross difference in affinity for the fluorescent stain was a peculiarity of malignant tissue or an expression of rapidly proliferating tissue the staining characteristics of regenerating rat liver were investigated, and the results are presented here.

Methods and Materials

Rabbits immunized with rat liver particles by the method of Weiler (1956*a*) were bled, and their serum was fractionated by precipitation with half-saturated ammonium sulphate solution at 0° C. The resultant globulin fraction, after dialysis against phosphate-buffered saline pH 7·3, was conjugated with fluorescein isocyanate by the method of Coons and Kaplan (1950). The resultant fluorescent rabbit globulin was dialyzed for five days and then absorbed with freshly prepared and acetone-dried kidney particles.

Adult Sprague Dawley and Wister rats weighing 150 to 200 grammes were subjected to partial hepatectomy using the standard method of removal of two lobes in which approximately two-thirds of the liver is removed. Rats were sacrificed under ether anæsthesia at twelve hours, and at one, two, three, four, five, six and seven days after operation. A small piece of liver was fixed on each occasion in Bouin's fluid containing 2% chromic acid and stained with hæmatoxylin and eosin after paraffin embedding. A similar piece of liver from the same lobe was snap-frozen in isopentane at −70° C., and frozen sections were cut at 5μ by a method already described (Louis, 1957). Similar sections were prepared from the margin of a hepatoma induced by feeding 0·06% 4-dimethylaminoazobenzene in a 20% casein diet containing two milligrammes of riboflavine per kilogram. The sections were mounted on clean slides, dried, immersed in absolute ethanol at −20° C. for 15 minutes, again dried and then stained for half-an-hour with the fluorescent stain. After washing for 10 minutes in three changes of buffered saline the sections were examined under the fluorescence microscope.

Results

At all stages of regeneration the rat liver showed even and bright fluorescence of the cytoplasm of the parenchymal cells. A section of rat liver taken 36 to 72 hours after partial hepatectomy shows well-developed fluorescence (Figure I). A section of a piece of the same lobe stained in a routine manner, for example, with hæmatoxylin and eosin, shows mitotic figures and binucleate cells indicative of an actively regenerating liver (Figure II).

In contradistinction to the actively regenerating liver, the neoplastic liver (Figure III) showed no fluorescence. The structure of the non-fluorescing areas is shown in a serial section stained in the routine manner (Figure IV).

Discussion

Study of the livers of rats which have been fed various hepatotoxic substances (including carcinogens) shows that the nodules of liver tissue, occurring in the subsequent cirrhosis, stain as well with fluorescent rabbit globulin as does the normal tissue. This suggests that hyperplastic tissue is essentially similar to normal and different from neoplastic tissue. It is possible, however, that a change in the liver cells, present at the time of maximal cellular division, has disappeared, and that these have reverted to normal when the tissue is not effectively subjected to the stimulus which previously caused cellular proliferation. To overcome this difficulty tissue was examined during actual cellular activity.

Regeneration of the liver after partial hepatectomy is rapid. Within 36 hours of partial removal there are easily demonstrated mitotic figures, multinucleated cells and large cells. Mitotic figures constitute only about 1% of the cells observed, and it is possible that the failure of these to fluoresce might be overlooked. The changes, of course, occur diffusely thoughout the organ and are not related to the site of injury. However, observations of the tissue stained with fluorescent globulin at low to moderate magnifications show no difference from the normal, and examination under high power does not show any poorly-staining cells.

The multinucleated cells are, in general, those which have recently undergone mitosis of the nuclei, and the larger cells are also evidence of altered activity or impending division. These altogether constitute, in the early stages, a considerable proportion of the cells in any field. It is improbable that the protoplasmic pattern is so unstable that evanescent changes involving temporary disappearance of protoplasmic components would occur. The observations that the tissue is, from the point of view of this staining technique, normal can therefore be accepted as significant, and also that the number of cells actually involved, recently affected or about to be involved in the proliferation at the time of examination is sufficiently large not to be overlooked or ignored. Such an interpretation is strongly

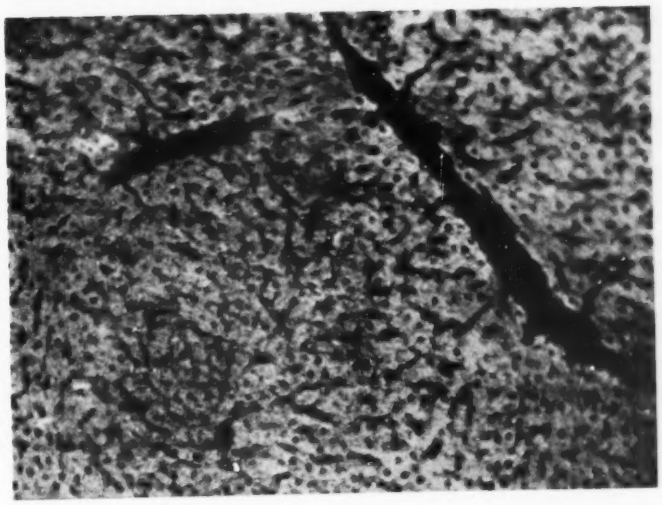

FIGURE I

Regenerating rat liver 72 hours after partial hepatectomy. Frozen unfixed section stained with fluorescent rabbit globulin showing even fluorescence of the cytoplasm of all parenchymal cells. (×160)

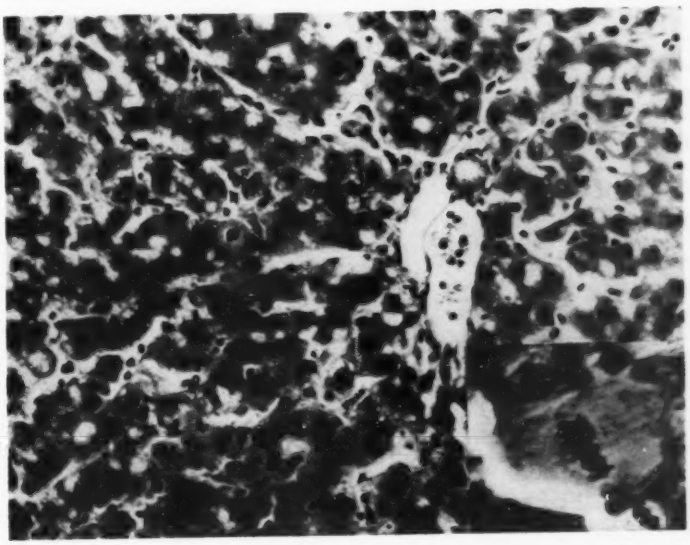

FIGURE II

Regenerating rat liver 72 hours after partial hepatectomy. Section taken from same lobe as Figure I. Stained with hæmatoxylin and eosin showing mitotic figures, binucleate cells and large cells (×240). Inset: Cell showing mitotic figure. (×950)

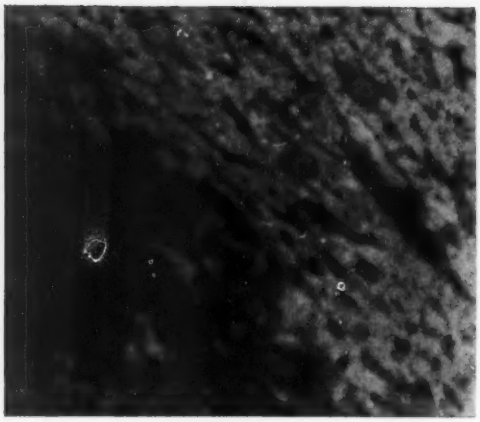

FIGURE III

Margin of rat hepatoma. Unfixed frozen section stained with fluorescent rabbit globulin. Only cytoplasm of innocent cells stains. (×120)

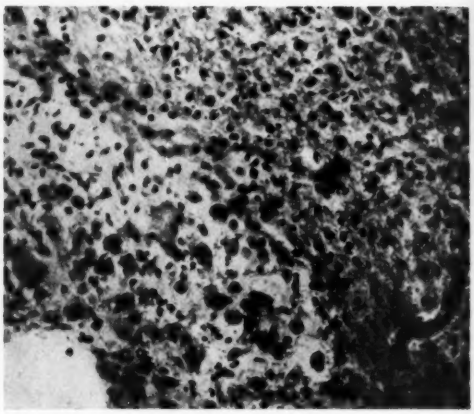

FIGURE IV

Margin of rat-hepatoma serial section to Figure III. Formalin fixed after cutting and stained with hæmatoxylin and eosin for comparison with section of Figure III. (×120)

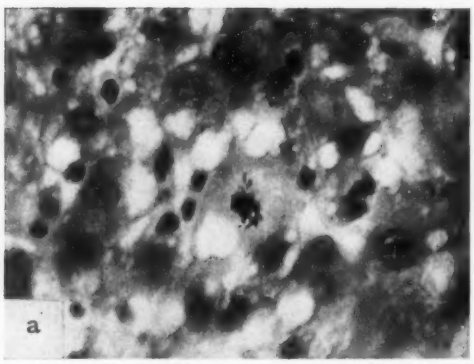

FIGURE VA

Regenerating rat liver 48 hours after partial hepatectomy. Section stained with hæmatoxylin and eosin showing large cells and mitotic figures. (Note mitotic figure in middle of field) (×750)

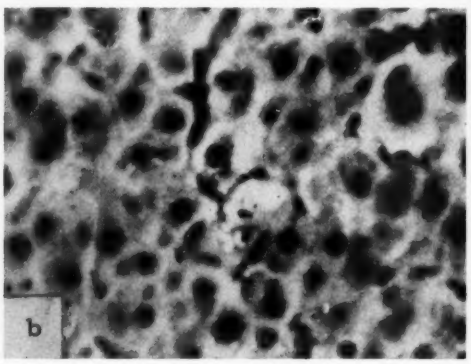

FIGURE VB

Regenerating rat liver 48 hours after partial hepatectomy. Fluorescence photomicrograph of the same section as Figure VA prior to fixation. All cells are fluorescing, but not the nuclei. The cell near the middle of the section is fluorescing well, and the scattering of light rays almost completely obscures the dispersed chromatin. (×750)

supported by the actual observation of an occasional fluorescing cell which subsequently has been shown, after hæmatoxylin and eosin staining, to be undergoing mitosis (Figure V*a* and *b*).

The staining reactions of regenerating liver are thus similar to those of normal liver, and both these have been shown to differ greatly from those of hepatoma cells. This difference in the staining of malignant tissue is thus not merely a function of its rapid rate of growth. As pointed out previously, this staining does not seem to depend solely upon an antibody-antigen reaction but probably reflects an alteration in the chemistry of the cell.

Creech and Jones (1941) studied the conditions for conjugation of aromatic isocyanates to protein and concluded that the ε-amino groups of lysine were probably involved with formation of carbamido linkages—that is to say, the basic groups of the proteins are involved. Electrophoretic studies of the soluble proteins of rat liver and rat hepatoma by Sorof and Cohen (1951), Eldredge and Luck (1952) and by de Lamirande, Allard and Cantero (1951) have shown that at an alkaline pH there is a significant decrease in the slowly moving protein fractions of neoplastic tissue as compared with normal, regenerating and pre-neoplastic liver tissue. However, Hoffman and Schechtman (1952), using a different method of extraction, found a diminution of the more slowly moving fractions during rapid regenerative growth.

Proteins with a low mobility under electrophoresis at pH 8·1 to 8·6 are amongst the most basic of liver proteins and could be expected to have a high affinity for any free fluorescein isocyanate in the stain. It is tempting to correlate the observed staining reactions with these alterations in proteins as determined electrophoretically. It is probable that unconjugated fluorescein derivatives are involved in the staining reaction, because complete removal of these derivatives by repeated absorptions with acetone-dried mouse-liver particles greatly diminishes fluorescence staining. Whatever the precise or fundamental changes occurring in the tumour cells during carcinogenesis, the significant point here is that malignant cells differ, in some particulars, from both the normal and physiologically proliferating cells.

CONCLUSION

The loss of affinity for one fluorescent stain observed in rat-hepatoma tissue is an important feature distinguishing this from the normal; it appears to be a demonstration of altered cellular chemistry coincident with the development of malignancy and not merely an expression of rapid cellular proliferation.

ACKNOWLEDGEMENT

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THE HISTOLOGY OF LOCALIZED EMPHYSEMA[1]

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SUMMARY

The histological features of several types of lesions showing localized emphysema are presented. Most such lesions were associated macroscopically with either bronchiectasis (or bronchiolectasis) or " scarring " in the affected area ; examples of each type are described.

In several of these localized lesions it was possible to demonstrate unequivocally that the emphysematous region was wholly aerated by collateral ventilation and that the original bronchiole that supplied the area was obliterated. These observations provide conclusive morphological evidence to support the hypothesis of pathogenesis of emphysema which was presented to account for the histological changes observed in the generalized condition, in which the evidence for the hypothesis was, to a large extent, deductive.

The close relation between the localized and the generalized disease is emphasized by the observation that bronchiolar inflammatory damage and obliteration is similar in pattern and the initial lesion is centrolobular (or " focal ") in both. The features of any emphysematous lesion, either localized or generalized, are shown to be determined largely by the nature of the causative inflammatory process, the emphysema itself being due to a single process—" trapping " of collaterally ventilated air.

This study of localized lesions also emphasizes the intimate association of the three manifestations of permanent inflammatory damage to the lung—emphysema, bronchiectasis and " scarring " ; it also indicates that these three are properly regarded as sequelæ of inflammatory obstruction and obliteration of bronchioles.

> Man is in truth ever the same ; even when the direct succession of cause and effect does not come in, we see that in times and places most remote from one another like events follow upon like causes.
>
> E. A. FREEMAN : " Comparative Politics ".

ALL classifications are necessarily arbitrary. On macroscopic appearances, emphysematous lesions were classed as either localized (in which most lung segments were unaffected) or generalized (in which every segment was involved). The generalized condition often showed local accentuation of the emphysema identical in character with localized lesions (McLean, 1956*a*). The histology of " purely " generalized emphysema has been discussed previously (McLean, 1957*a*, *b*). Here it is proposed to consider localized disease and the closely related lesions superimposed on generalized emphysema.

It is clear that localized lesions may progress to a greater degree than the generalized condition, since adequate pulmonary function can be maintained by the remaining normal lung tissue. For this reason the localized variety of emphysema usually becomes apparent clinically only in the extreme form of large discrete spaces, often termed air cysts. Although much has been written in recent years on the clinical and radiological aspects of the cystic forms of emphysema, and their pathogenesis has been discussed with enthusiasm, little study has been made of their morphological evolution.

This presentation is concerned largely with the early, and therefore less morphologically complex, forms of the localized disease. The aims of this study are to support and extend the hypothesis of pathogenesis of emphysema propounded previously (McLean, 1957*a*, *b*), to demonstrate the factors determining the development of localized lesions and to emphasize the close relation of the three morphological sequelæ of bronchiolar inflammatory disease—emphysema, " scarring " and bronchiectasis.

MATERIALS AND METHODS

Techniques, along with much of the material, have been described previously (McLean, 1956*a*). Other material used here included approximately ten blocks of localized emphysematous lesions that were serially sectioned in part.

[1] Received on August 27, 1956.
[2] Research Fellow, Department of Pathology.

RESULTS

Macroscopic examination of examples of localized emphysema indicated that most were associated with some obviously related lesions such as ectatic bronchi or bronchioles or pulmonary "scars" (McLean, 1956*a*). Observations on the histology of these lesions may conveniently be incorporated in the same classification.

Localized Emphysema and Bronchiectasis

Although satisfactory generalizations may be made about this group as a whole, many of the features can be more clearly presented and the complicated relations elucidated by considering special illustrative examples.

One such lesion contained a dilated bronchus which led to an emphysematous subsegment, the remainder of the lung being normal. Examination of serial sections through this bronchus showed that there were only two small peripheral communications with the surrounding lung.

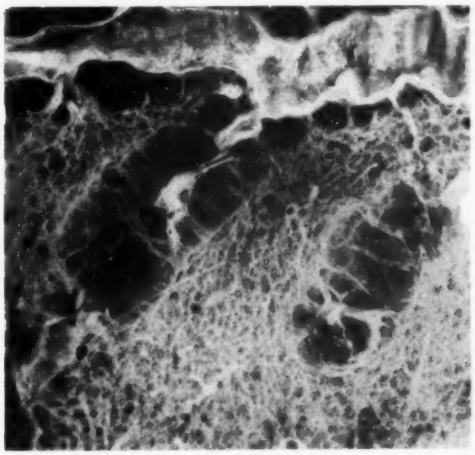

FIGURE IA

Photograph showing a dilated bronchiole (above) which, with its branches, is surrounded by a zone of emphysema. (×5)

Obviously most of the original branches of this bronchus had been obliterated, but, as was usually the case in such circumstances, remnants of individual obliterated branches of the bronchus could not be recognized.

The interrelation of ectasia, bronchiolar obliteration and emphysema was demonstrated very much more clearly in a specimen in which a narrow branch of a much dilated bronchiole was surrounded by localized emphysema (Figure I). The walls of both bronchioles contained little smooth muscle or elastic tissue. The narrow branch divided several times, and two of its divisions were traced in serial sections. Both of these extended laterally towards the emphysematous spaces along with their attendant pulmonary arterioles. A short

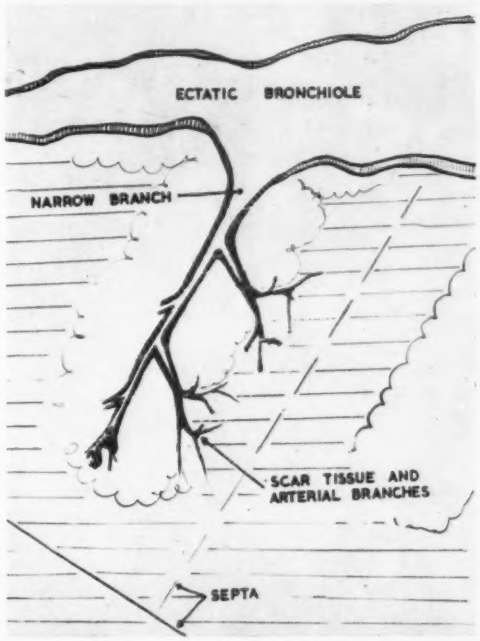

FIGURE IB

Diagram of reconstruction of sections through the region, one side of which is depicted in Figure IA. Leading from the narrow branch two bronchioles are shown; both end blindly, and scar tissue continues along the arterial branches into the normal lung, which, with the adjacent emphysematous area, is aerated by collateral ventilation

distance from their origin both bronchioles narrowed progressively, their epithelium becoming simpler, until they ended blindly (Figure II). In each case collagenous connective tissue continuous with that forming the bronchiolar wall extended along with the accompanying arteriole into the emphysematous region. Approximately at the site where the bronchiole finally disappeared, the surface of this collagenous cord was lined, where it formed part of the wall of the emphysematous spaces, by cuboidal epithelium. Closely applied to the arteriole and dividing with it, this cord was traced out farther, finally traversing the

emphysematous area and ending in relatively unaffected lung. From the terminations of this scar recognizable alveolar ducts extended yet farther out, branching normally (Figure III). Reconstruction of the emphysematous spaces gave no indication of their original structure; all spaces immediately related to the wall of the narrowed bronchiole and its divisions intercommunicated freely.

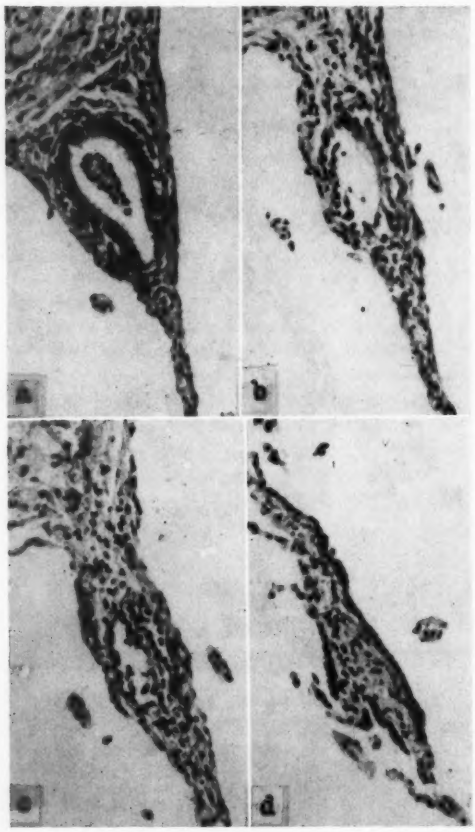

FIGURE II

Photomicrographs showing sections through the upper obliterated bronchiole in Figure IB. The bronchiole is initially lined by ciliated epithelium, which becomes indeterminate near the site of obliteration, 300μ and 320μ peripherally. At 340μ the lumen disappears, and at 420μ the outer surface of the residual connective tissue cord is lined by cuboidal epithelium. (×180)

The dilated bronchiole from which this narrow branch with its obliterated divisions arose showed, farther distally, a similar narrowing, and most of its divisions were also obliterated, only one passage being traced distally to open into dilated air spaces. The epithelium lining the dilated part contained many goblet cells, but they were not found in the epithelium lining the narrow passages branching from it. Arterial branches showed considerable intimal sclerosis as far as the arterioles accompanying the terminations of the epithelium-lined passages (or the scar tissue in the case of obliterated bronchioles).

In this example it was evident that the original bronchiolar passages leading to the emphysematous spaces had been obliterated and

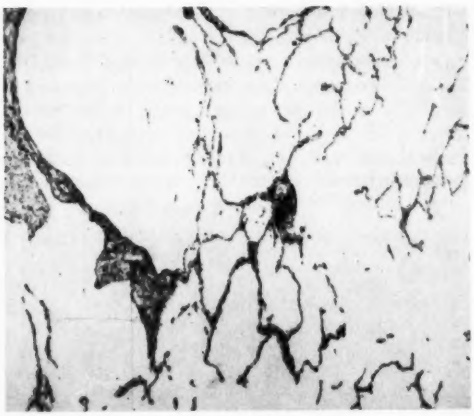

FIGURE III

Photomicrograph showing the lower obliterated bronchiole of Figure IB (as a small passage in scar on left) just proximal to the site of obliteration. Scar tissue extends peripherally through the emphysematous region to end in relation to relatively unchanged passages resembling alveolar ducts. Much of the scar is lined by epithelium on its surface. (×22)

that subsequently the dilated air spaces must have been aerated by collateral ventilation from adjacent lobules. More often, however, even in similar small localized lesions, such as surround damaged bronchioles in bronchiectatic lobes (Figure IV), this relation could not be demonstrated, because the obliterated bronchioles could not be identified even in the walls of bronchioles which ended blindly.

In generalized emphysema, localized accentuation of dilatation and destruction of air spaces were often seen affecting anatomical units of lung—usually subsegments, in which the bronchus or bronchiole was obviously considerably more dilated than in adjacent regions. This was common in emphysema associated with prolonged asthma. Communications of such bronchi or bronchioles with

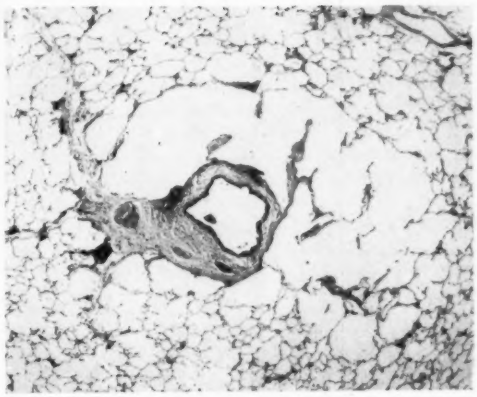

FIGURE IV

Photomicrograph showing an ectatic bronchiole surrounded, in part, by emphysematous air spaces which are traversed by strands of scar tissue. The wall of the bronchiole is extensively damaged, and the collagenous tissue surrounding it is continuous with that surrounding sclerosed pulmonary arterial branches. Away from the bronchiole the lung is normal. (×16)

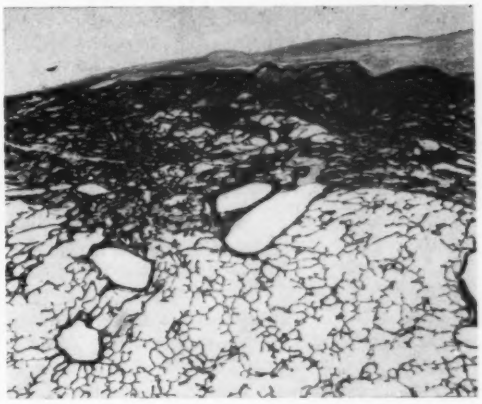

FIGURE V

Photomicrograph showing part of a typical flat apical scar. The scar consists of thickened pleura with a considerably greater amount of condensed organized lung, the distinction being obvious with elastin staining. A dilated bronchiole ends at the undersurface of the scar, and one of its obliterated continuations into the scar is readily distinguished. The underlying lung is normal. (×12)

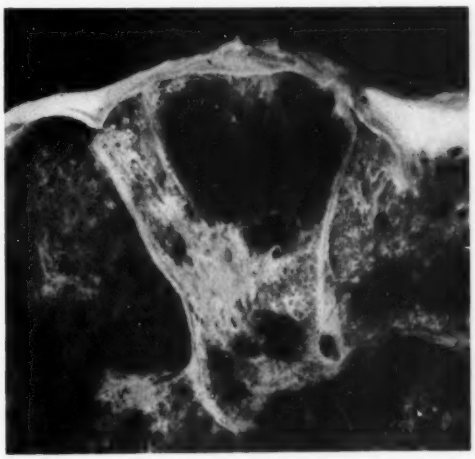

FIGURE VIA

Photograph showing, clearly demarcated by septa from relatively normal lung, a damaged unit of lung tissue mostly consisting of scar tissue but with an emphysematous region peripherally

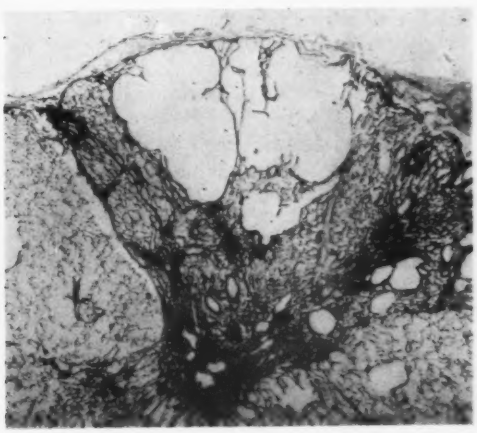

FIGURE VIB

Photomicrograph of a section from the same block of tissue. Serial sections revealed that the bronchiole originally supplying this region and that adjacent (right) were obliterated. (×4)

the emphysematous spaces were fewer than those arising from similar passages in less emphysematous areas.

Localized Emphysema and "Scars"

Macroscopically localized emphysema was commonly found adjacent to "scars" in the lung. Lung "scars" showed certain histological features regardless of their size or situation. Certain of them were not associated with emphysema in adjacent tissue, and this group, because of the minimal distortion of adjoining lung, illustrates well the features of lung scars. Generally, flat apical scars were of this type. Sections of these, stained with hæmatoxylin and eosin, showed a dense uniform scar; leading to the scar were relatively large (and often dilated) bronchioles which either ended blindly at the edge of the scar or, dividing into tortuous narrow passages, penetrated only for a short distance. Occasionally these passages led to surviving aerated spaces within the scar, but more often the lumen tapered and was obliterated, usually surrounded at this site by an accumulation of lymphocytes.

As Bunting (1942) pointed out, elastic stains often reveal the original structure of tissue forming the scar, the amount of elastic tissue varying from relative abundance (Figure V) to only scattered fibres. In large scars residual muscle fibres were found. With careful examination evidence that a "scar" represented what was originally a much larger volume of lung tissue could always be obtained; this also applied to subpleural scars, which often included, in addition, some thickened pleural tissue (Figure V).

Scars associated with emphysema, particularly where this was gross, were usually considerably distorted. The original structure was still partly recognizable in one example (Figure VI), in which a dilated large bronchiole led to a scarred area at the apex of an upper lobe, ending blindly within the scar. By reconstruction of serial sections it was possible to show that the emphysematous area, which was still enclosed by recognizable septa (Figure VI), was originally supplied by a branch of this bronchiole, but that this branch was not obliterated. No patent bronchiole led to the region, and the remnants of divisions of the obliterated bronchiole could be traced distally to end adjacent to distorted and dilated air spaces (Figure VII). The scar, representing the remainder of the tissue supplied by the bronchiole, surrounded the emphysematous region on three sides and also formed its base.

Although it is obvious in this example that air reached the emphysematous area by collateral ventilation, such clear examples were uncommon for two main reasons: first, obliterated bronchioles were usually more difficult to identify, and secondly, with enlargement of the emphysematous region, septa between lobules were often destroyed and the region appeared to be supplied directly by neighbouring patent bronchioles. Only in the earliest lesions, where the original lobular structure could be defined, was it possible to demonstrate that such bronchioles did not originally supply the emphysematous region.

These points are illustrated in an example of slight emphysema surrounding a small tuberculous lesion four millimetres in diameter. The fibrocaseous nodule contained no specialized tissue but, since it was continuous proximally with the wall of a bronchiole and was intimately related to an arterial branch, the tuberculous process had clearly involved and obliterated a large bronchiole. The emphysematous spaces surrounding the area, however, freely communicated with adjacent normal lung; only by careful reconstruction was it possible to obtain evidence that these spaces were originally supplied by the obliterated passage. With lesions larger than these and with techniques appropriate to and suitable for the smaller lesions, such reconstructions were impracticable.

CYSTS AND BULLÆ

Macroscopic discrete cystic air spaces were commonly found both in otherwise "normal" lungs and in lungs showing different degrees of generalized emphysema. The structures most readily studied histologically were small single cysts within the parenchyma of macroscopically normal lung. These air cysts varied from lung to lung in size and position. Those studied were mostly divided into cysts within the lung parenchyma and subpleural cysts. In each case cysts were either single or multiple.

Small cysts within the lung parenchyma were frequently encountered; many were surrounded by macroscopically normal lung tissue, and of these few were more than 1·5 centimetres and none was over 2·0 centimetres in diameter. The smallest cysts closely resembled the lesions of focal emphysema (McLean, 1957*b*), in several examples of which some or many of the foci had the appearance of small "tension cysts". Some of these were studied histologically in an attempt to find morphological variants to account for the cystic change. In the larger cysts the communications with the surrounding unaffected peripheral air passages were narrow

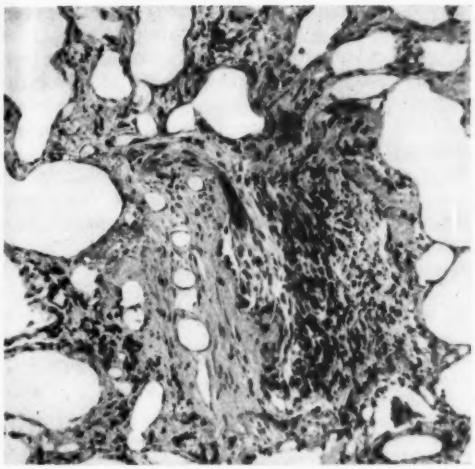

FIGURE VIIA

Photomicrograph showing, near the emphysematous part in Figure VI, the remnants of a branch of the obliterated bronchiole supplying the damaged area. A small dark area (above centre) could be seen, under higher magnification, to be a group of epithelial cells. Adjacent is a mass of cells containing old blood pigment (right). Hæmatoxylin and eosin. (×90)

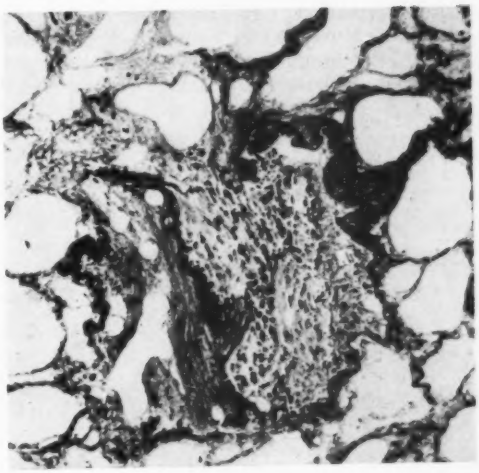

FIGURE VIIB

Distribution of degenerated elastic tissue suggests the outline of the original bronchiolar lumen. Elastin stain. (×90)

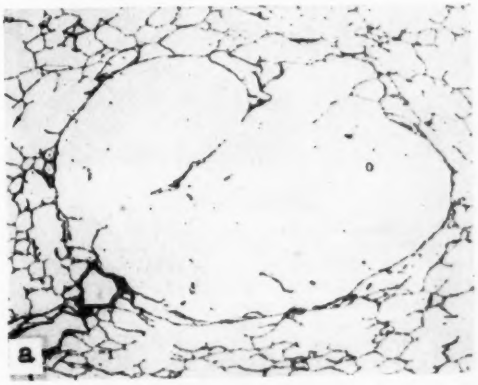

FIGURE VIIIA

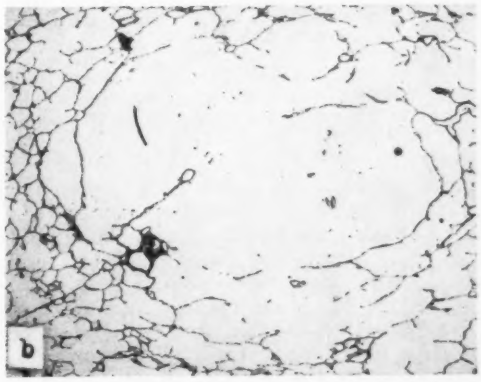

FIGURE VIIIB

Photomicrographs showing one of a large number of small air cysts in a lung—a form of focal emphysema. This cyst was supplied by a bronchiole which is shown near its origin (Figure VIIIA) and, after turning to traverse 600μ in the wall of the cyst (Figure VIIIB), just proximal to the site at which it opened into the cyst. Residual strands of tissue containing small arterial branches are shown crossing the cyst. (×16)

and tortuous, either because of compression of the surrounding lung or extensive connective tissue formation and deposition of black pigment. However, in some of the smaller cysts the communications were free and unrestricted, although adjacent lobules usually

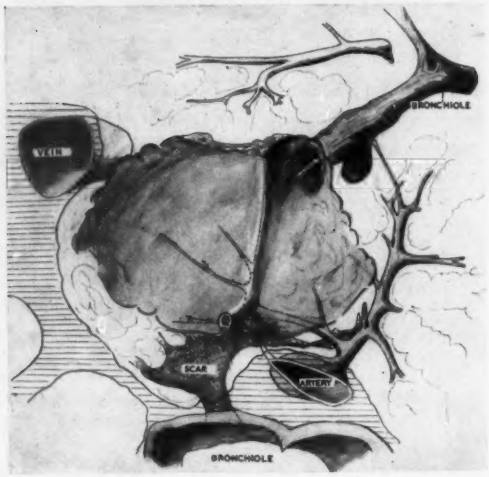

FIGURE IX

Diagrammatic reconstruction of the relations of a small air cyst in a lung showing only slight histological emphysema elsewhere. That part of the cyst not illustrated was limited by extensions of the septa shown. A bronchiole communicates directly with the cyst, but the original bronchiolar supply of the area is represented only by a scar which ramifies in association with arteries distinct from those related to the patent bronchiole (above)

showed slight focal change. Three such lesions were specially examined, and in each a bronchiole opened into the space. This bronchiole was two to four divisions distal to the bronchioles supplying the lobule and in each case showed some evidence of old inflammatory damage. In these respects the bronchiolar morphology did not differ from that found in early focal emphysema. However, in each of these cystic lesions the bronchiole opening into the cyst formed part of its wall, entering it at some distance from the site at which it first came into contact with it (Figure VIII). In one example an irregular strand of connective tissue was traced from the bronchiolar wall, accompanying a small pulmonary arterial branch for a short distance across the cyst, the vessel supplying normal tissue on the side remote from the bronchiole. In the others structures suggestive of obliterated bronchioles were not seen.

Isolated cysts within macroscopically normal lung were often more complex. The structure of one example will be presented in detail; this cyst was 9·0 millimetres in diameter, and on histological examination the surrounding lung showed slight focal emphysema (lung lobules surrounding cysts of this size or larger always showed some evidence of old inflammatory damage). Reconstruction of serial sections through the area (Figure IX) showed that, at one side, a bronchiole entered the cysts at a somewhat oblique angle. The communication between the bronchiole and the cyst appeared relatively free, with no suggestion of any "flap-valve" over its orifice. The tissue forming the wall of the cyst was compressed, and therefore the communications of the cyst with the surrounding lung were tortuous. In the wall of the cyst, bulging into the lumen,

FIGURE X

Photomicrograph showing some of the features of the cyst illustrated in Figure IX. Part of the scar is shown near its origin from the wall of the bronchiole (below). The artery to which the scar is related is sclerosed. The patent bronchiole which communicates with the cyst is shown (above) near the site of communication. Elastic stain. (×10)

was a pulmonary arterial branch which showed considerable narrowing of its lumen due to intimal thickening; some arterioles arising from this vessel supplied parts of the lung adjacent to the cyst (Figure IX). This artery arose from a vessel accompanying a large

bronchiole which showed much evidence of old inflammatory damage; at one site, near the cyst, there was little specialized tissue in its wall, and a mass of scar tissue led from this site to become related to the damaged artery (Figure X). This collagenous tissue was traced peripherally, dividing with the arterial divisions (to which it was intimately related). In one area an arterial branch, accompanied by scar tissue, led outside the cyst to relatively normal peripheral passages which clearly did not take origin from any existing patent bronchiole (Figure IX).

Other cysts wholly within the lung were sometimes related to macroscopically visible scar tissue which was sometimes extensive. In cysts in lungs showing diffuse "pulmonary fibrosis", although communications could always be traced between the cysts and surviving

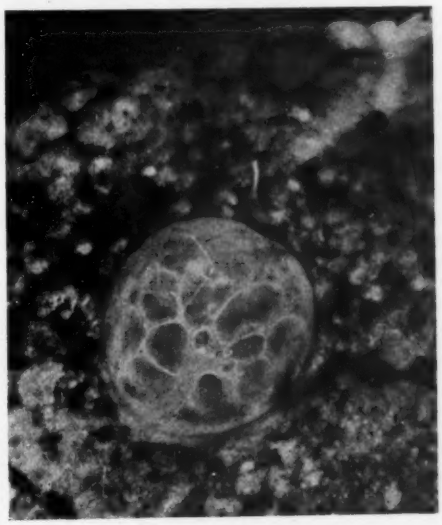

FIGURE XIA

Photograph showing a small subpleural air cyst in the lung of a patient who had had chronic spasmodic asthma and whose lungs showed moderate diffuse emphysema. (×2)

bronchioles, it was never possible to determine the original bronchiolar supply to the emphysematous area, because of the gross structural changes and extensive bronchiolar obliteration. Unlike cysts in macroscopically normal lung, in diffusely scarred lungs, some examples which were largely surrounded by scar tissue, were over 2·0 centimetres in diameter.

Subpleural cysts (frequently referred to as bullæ), unlike cysts within the lung, were often of large size. Most such cysts had a narrow pedicle, and it was clear that the enlargement resulted mainly by stretching of the pleural layer. In otherwise macroscopically normal lungs most subpleural cysts (or bullæ) were found at the apices, where, almost without exception, they were associated with an apical

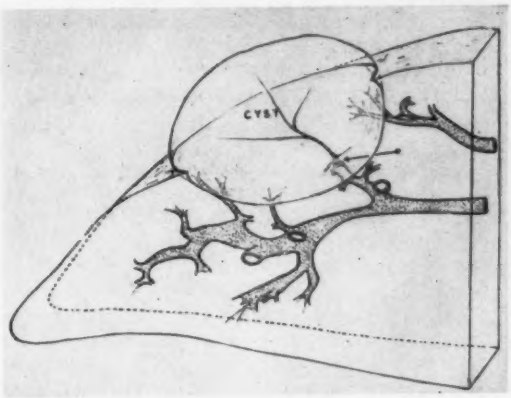

FIGURE XIB

Diagrammatic representation of the bronchioles related to the cyst, the variation in calibre being remarkable. Four groups of bronchiolar divisions are related to the cyst, but, although none opened into the cyst, of these only one (indicated by arrow) originally supplied the tissue, which is now cystic. Scar tissue continuous with the wall of this bronchiole extends into the cyst.

scar. These cysts appeared to evolve from lesions similar to that depicted in Figure VI, and, despite the common macroscopic impressions, the scar at the base was not simply due to compression of underlying lung.

Occasionally subpleural air cysts were found, at the base of which there was little scar tissue. Most of these were found in lungs showing a severe degree of macroscopic generalized emphysema. They varied greatly in size, from 1·0 to 10 centimetres in diameter. One small cyst, 1·7 centimetres in diameter, was found in the right lower lobe of a middle-aged patient who had had spasmodic asthma since childhood (Figure XI). Serial section through this cyst included all bronchioles related to it and to the lung immediately surrounding it. All but one of these bronchioles supplied lung tissue outside the cyst, although the ramifications of some of them ended in compressed air passages forming the wall of the cyst. The remaining bronchiole, which showed even more evidence of old inflammatory damage, differed from the others in that, although its irregular branches did not

enter the cyst directly, a cord of connective tissue continuous with the walls of the distorted bronchiolar divisions (Figure XII) entered the cyst along with an arteriole. Where it entered the cyst this connective tissue cord was lined on its outer aspect by epithelium. The arteriole traversed the cyst, becoming smaller and smaller with few recognizable divisions, one fine vessel supplying tissue on the side of the cyst opposite from the bronchiole. The collagenous tissue cord entering the space was probably an obliterated bronchiole because of its origin in the wall of a damaged bronchiole and its relation to

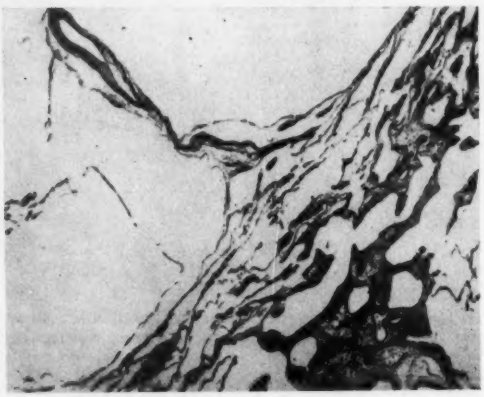

FIGURE XII

Photomicrograph showing the region in which the bronchiole, depicted in Figure XIB, terminates. The bronchiole opens into a number of tortuous spaces lined by flat epithelium, between which there is much scar tissue, containing some elastic tissue. This scar tissue was continuous (in other sections) with that accompanying the small artery (upper left) and its branches within the cyst. (×22)

the arteriole traversing the space. If so, it would have been the bronchiole which once supplied the emphysematous region, since no other bronchiole or remnants of bronchioles were related to the cyst.

Although, in this particular lesion, more definite proof that the collagenous strand was an obliterated bronchiole could not be obtained, it was the largest air cyst examined in which no bronchiole directly entered the emphysematous region. In large cysts there was even greater distortion of structure, and one or more bronchioles had a more or less direct communication with the air space; in these it was not possible to gain any satisfactory conception of the original relation of the emphysematous region to these bronchioles.

DISCUSSION

The hypothesis of pathogenesis of generalized emphysema propounded previously (McLean, 1957*a*, *b*) was based on the conclusion that the air passages beyond an obstructed bronchiole can remain aerated by collateral ventilation and that this air enters more freely than it leaves the area. If collateral ventilation is sufficiently reduced, air-trapping results, producing dilatation of peripheral air passages and disruption of their walls. (By peripheral air passages is meant the distal respiratory bronchioles and the alveolar ducts; the aveoli are best regarded as individual diverticula of these passages. Dilatation of these passages causes the alveoli to become relatively shallow; disruption of the walls of the passages results in disruption not only of alveolar walls but also of the musculo-elastic mesh forming the main passage.)

In some of the localized lesions described here it has been possible to demonstrate clearly emphysematous change in peripheral air passages that had been originally supplied by bronchioles that were now shown to be obliterated; so that the aeration of the emphysematous regions must have been dependent on collateral ventilation. In the generalized condition the evidence for the hypothesis is strong but is only circumstantial, the topographical relation of obliterated bronchioles to the peripheral passages once supplied being rarely demonstrable.

The hypothesis demands that significant localized air-trapping occurs only with either gross bronchiolar obliteration or reduced freedom of collateral ventilation, or both simultaneously. This is illustrated clearly in localized lesions.

It was demonstrated first in the group of lesions associated with localized bronchiectasis or bronchiolectasis. These dilated passages always showed extensive obliteration of their more distal divisions; indeed, any degree of dilatation of such a passage was always associated with considerable, or even occasionally total, obliteration of the distal passages. In these lesions it could be shown easily that the emphysematous lung was originally ventilated by bronchioles that had become obliterated but was now ventilated through collateral channels from the adjacent normal lung. In other forms of emphysema this point is more difficult to demonstrate.

Peripheral air passages in aerated lung surrounding ectatic bronchi or bronchioles always show some emphysematous change. The degree of this change depends on the extent of collateral ventilation in the area. In many cases therefore it is only those peripheral passages

farthest removed from the source of air supply that are emphysematous (Figure I).

Secondly, the factors producing localized air-trapping can also be demonstrated most clearly in lesions associated with obvious scar tissue. The emphysema may be extreme in these lesions. The original bronchiolar supply to these emphysematous regions is obliterated; presumably, this obliteration occurs during the same inflammatory episode or episodes as those in which the "scar" forms. In the area now represented by scar, exudate entered the distal passages, and, after occlusion of their alveolar pores, air was absorbed and they became airless and, later, organized. The "scar" is further significant in that it restricts the freedom of collateral ventilation to the emphysematous area; in macroscopically normal lung air-trapping in the passages beyond an obliterated bronchiole will become significant only if the normally free collateral ventilation is limited, because much of the immediately adjacent tissue is impervious to air. This is often the situation at the apices when collaterally ventilated tissue is largely enclosed by scar below and pleura above. The same situation is uncommon in the lung parenchyma, but may occasionally be seen in patches of "diffuse pulmonary fibrosis" ("scarring").

Scars are the end result of many different inflammatory processes—all types may be accompanied by emphysema. Unlike generalized emphysema, in which the bronchiolar damage is due to generalized inflammations—most of which are viral in origin with secondary infection by the bacteria of the naso-pharynx (McLean, 1957*c*)—localized scarring and emphysema are often due to local conditions, of which tuberculosis is the most important. Opie and Aronson (1927) considered that most apical scars were tuberculous in origin, but Bunting (1942) has pointed out that there is no evidence of a tuberculous origin in most of the very common flat apical scars. Apical cysts may also occasionally be found in association with local obstructive lesions, such as neoplasms complicated by non-specific bacterial infection (Castex and Mazzei, 1951).

Local accentuation of emphysema with formation of an air cyst is a common condition. Such air cysts are often subpleural, in which situation they are usually termed "bullæ". Bullæ must be distinguished from blebs which are due to acute interstitial emphysema where air enters the connective tissue of the lung (Miller, 1926, 1927). In the present study no evidence was obtained that there was such a condition as chronic interstitial emphysema; what appeared macroscopically to be small blebs were either obviously of recent formation or, more usually, could be shown to be dilated natural air passages—that is, they were bullæ. Other terms are used to describe air cysts—pneumatocele, giant cyst and balloon cyst; these terms are largely interchangeable.

Authors describing air cysts have usually confined their attention to one particular condition in which air cysts are found. Most reports have been concerned with "congenital cystic lung", with large air cysts developing in the course of respiratory infections in infants or with the bullous form of emphysema. These reports have been essentially clinical or radiological, but discussion of ætiology has been rarely omitted. Of the hypotheses offered, most favour the concept of "ball-valve" obstruction of bronchi or bronchioles. This concept of ball-valve obstruction is based on the observation that bronchi and bronchioles increase in diameter on inspiration and decrease with expiration. A partially obstructed passage might therefore permit air to enter during inspiration but the passage be blocked on expiration. An older but similar concept held that loose material, such as mucus, could move up and down the bronchial tree, producing a more literal ball-valve effect—few seriously consider that such a phenomenon could persist beyond one (or perhaps two) respiratory cycles. The pathological evidence for these bronchial or bronchiolar lesions is dubious. This is admitted by some authors, such as Caffey (1940), but others repeatedly quote the work of Hayashi (1914), who found partial obstruction by connective tissue of bronchioles leading to cystic air spaces. Such lesions are relatively uncommon and were seldom demonstrable in any of the patent bronchioles near small cysts. Proponents of ball-valves in bronchioles might suggest that the partial obstruction is due to spasm or mucosal swelling. Such phenomena are of a temporary nature, and accumulated secretion would rapidly produce complete obstruction in the smaller air passages. It is difficult therefore to imagine such a lesion persisting in a small bronchiole for hours and certainly not for years.

There are other objections to placing significance on bronchial or bronchiolar "ball-valve" obstruction; some have been presented previously (McLean, 1957*c*). Apart from morphological considerations there is a strong objection on physical grounds. The most efficient ball-valve cannot do more than dilate passages, which retain their normal elasticity, to the position of inspiration; once this degree of dilatation is reached, no more air can enter. Normal, but compressed, lung forms the wall

of some small cysts; the elasticity of the lung surrounding the cyst is patently normal, so that the distension of the cyst could not have been caused by a ball-valve obstruction in the supplying bronchiole. The only simple explanation remaining is that air must have been forced into the cyst during expiration (or coughing), the air being trapped in the cyst through the inspiratory phase of the respiratory cycle. This could occur only if the supplying bronchiole were completely blocked—either collapsed by pressure in the cysts or permanently obliterated, the air entering by collateral ventilation through peripheral valve-like air passages in the wall of the cyst.

Of all those who have written on pulmonary air cysts only Head (1951, 1955) has expressed similar views and reached similar conclusions. He described two cases in which one lobe was grossly emphysematous, compressing the adjacent lung. In both cases the appropriate bronchus was completely obliterated near its origin, and aeration of the emphysematous lobe depended on collateral ventilation across incomplete fissures. Another similar case is described, without discussion of the implications, by Falor and Kyriakides (1949). Head suggested that collateral ventilation of the affected lobes was restricted, so that air could enter only with coughing, the tissue becoming distended and emphysematous. He compared these cases with acute obstructive emphysema in infants, in which a definite bronchial "ball-valve" can be conclusively demonstrated to be the cause of the condition; in these patients the lobe inflates only to the volume it occupies in maximal inspiration (and therefore can only be visualized radiologically in expiration). In the more recent article (Head, 1955) the author extends the concept of trapped collaterally supplied air, admittedly with little further evidence, to account for all forms of emphysema.

The formation of air cysts can be followed by examining the smallest lesions. These differ only in size from examples of focal emphysema, which is indeed the early stage of all forms of emphysema—both localized and generalized. In the early focal or centrolobular lesions, air-trapping beyond an obstructed bronchiole is increased by the occlusion, with exudate or exogenous material, of many of the alveolar pores in the walls of the affected passages (McLean, 1957*b*). With expansion of the space and disruption of the walls communications develop with adjacent patent bronchioles. If these communications are simple and direct, as they are in most focal lesions, air-trapping is greatly reduced and dilatation and disruption cease. However, a length of the bronchiole sometimes forms part of the wall (Figure VIII), its course through this is oblique, and the communication becomes valvular. With strong expiration or coughing the rise of cyst pressure might collapse the bronciole. Such cysts would then enlarge further owing to the increased tension until disruption of the surrounding tissue led to the production of a more direct bronchiolar communication. This sequence would account for the transient nature of many of the large high tension cysts of recent formation such as have been observed following acute pulmonary infections in infancy and childhood, in which cysts observed in the acute stage may persist even for some months after the infection has resolved and then suddenly disappear (Caffey, 1940, 1953; Bloomer *et alii*, 1955). Cysts of longer duration which increase in size slowly, if at all, can be assumed to have reached an equilibrium of a kind, there being only sufficient air-trapping to maintain a slight elevation of pressure within the cyst throughout the respiratory cycle. It is not intended to suggest that all persistent air-containing cavities in the lung are maintained by trapping of air reaching the space by collateral ventilation. As, for instance, in most tuberculous cavities, the persistence of the cavity can often be demonstrated to be due to a true bronchial "ball-valve" mechanism produced by partial obstruction of a large bronchus by a stricture or tuberculous granulation tissue.

Where all divisions of a lobular bronchiole are obliterated but the surrounding lung is relatively normal, even the expansion of the cyst will be limited, because it will eventually encroach on and develop relatively direct communications with bronchioles in adjoining lobules (as in Figure IX). This would account for the apparent limitation of size of air cysts in normal lung parenchyma to about 1·5 centimetres in diameter, and also for the observations of Heppleston (1953) that most such cysts have a direct bronchiolar supply and that this communication with the cysts often appears valvular. Heppleston considered that this was operating as a bronchiolar "ball-valve", admitting air with inspiration and preventing it leaving with expiration. It has already been pointed out that this would not produce sufficient pressure; in any case, it does not explain those examples in which the bronchiole originally supplying the affected region can be clearly demonstrated to have been obliterated.

If much of the tissue around the cyst is dense and impervious to air, this will have two effects: first, the nearest patent bronchioles may be so remote anatomically that the collaterally ventilated area may still be

recognizable at a relatively advanced stage of development of the lesion (as in Figure VI), and secondly, this impervious tissue, be it pleura or scar tissue, will reduce collateral ventilation and therefore increase air-trapping. It is significant that several observers have sought, but did not find, direct bronchiolar communications with such subpleural bullæ (Loeb, 1930; Anspach and Wolman, 1933).

The largest cysts are subpleural, and in these the tissue at the base of the cyst is scarred. The basic changes leading to air-trapping—bronchiolar obliteration and reduced collateral ventilation—can be demonstrated. The cyst enlarges by stretching the pleura—a process which is relatively unlimited. At a certain stage the wall will be stretched beyond its elastic limit, and dilatation will proceed until there is a balance between intracyst pressure and intrapleural pressure on one hand and the amount of air entering the cyst and the amount being absorbed by the wall on the other (that some air absorption does occur is indicated not only by the observation of Beneke (1913) that the contents of such cysts is largely nitrogen but also by the general observations that air enclosed by tissue anywhere in the body is absorbed). Equilibrium may never be attained, and some such cysts continue to expand, and the patient dies with symptoms and signs identical with those produced by a tension pneumothorax. Such high-tension, rapidly expanding cysts are rare; since the pressure in bullæ is normally not much greater than in the rest of the lung, it is reasonable to assume that, with normal respiration, air-trapping in such bullæ is relatively slight, and that intracyst pressure increases only with coughing and subsequently declines to the original level. Head and Avery (1949), when examining the interior of subpleural air cysts with a thoracoscope, observed that, in some, air entered during inspiration, while in others it entered during expiration. The apparent paradox can be explained. The essential feature of air trapped in a region aerated by collateral ventilation, is that air enters the region more rapidly than it leaves. Where collateral ventilation is relatively free, air-trapping is slight; air entering with inspiration does not leave so readily. However, where the course of collateral ventilation is tortuous, air-trapping may be considerable; and if the peripheral air passages in the area (assuming their elasticity to be near normal) be distended beyond maximal inspiratory size, air cannot enter during inspiration, but enters only during expiration when the pressure in the adjoining normal lung rises above that in the emphysematous area. In the extreme case significant amounts of air may enter the area only during coughing. Thus the maximum pressure in a cyst, that can theoretically develop and be maintained, approximates to the intrapulmonary pressure during the phase of coughing before the larynx opens.

The interrelation of emphysema, "scars" and bronchiectasis (or bronchiolectasis) can be assessed by study of these localized lesions. All are sequelæ of acute bronchiolitis which has resolved incompletely. During the exudative phase of acute bronchiolitis the lung distal to the affected bronchioles (which are obstructed by inflammatory products) may or may not be involved in the inflammatory process (McLean, 1956*b*). If it is involved and becomes airless, it may not resolve, and a "scar" forms. Such a "scar" may vary in extent from a secondary lobule to a complete lung. In such "scars" bronchiolar obliteration is extensive.

If on the other hand the lung remains aerated (or becomes re-aerated), bronchiolar obliteration may still occur—although not to the same extent as in scars; aeration of lung distally is maintained by collateral ventilation. Significant air-trapping and, therefore, emphysema result where collateral ventilation to the area is restricted by excessive length and tortuousity of peripheral air passages through which this air must pass. In generalized emphysema this is largely the result of diffuse bronchiolar obliteration, but, in the localized disease, bronchiolar obliteration, even when gross (as it is in the distribution of ectatic bronchioles) does not produce significant emphysema unless collateral ventilation is further reduced by some other factor. The only exception to this would be found if there were extensive bronchiolar obliteration throughout the whole extent of a part of the lung having no ventilatory connection with the remainder—that is, a lobe with complete fissures. Lobar emphysema is rare but has been described (Fischer, Potts and Holinger, 1952; Shaw, 1952; Williams, 1952). In these cases the bronchi in the affected lobe were often ectatic, but no detailed histological examination of the small bronchioles has been recorded.

In areas of localized emphysema involving less than a lobe, restriction of collateral ventilation by tissues impervious to air can usually be recognized. The commonest form is the combination of pleura and "scar" tissue. Where such restrictions are not present, the emphysema is rarely gross even with considerable bronchiolar obliteration.

The pathogenesis of the dilatation of the bronchi and bronchioles, that is so often seen in emphysematous regions, has never been

satisfactorily explained. Ectasia of a bronchus or a bronchiole indicates that many or even all of its distal divisions are obliterated. Although this degree of obliteration is found often in passages leading to scars or larger amounts of condensed and organized lung such as chronically collapsed lobes, it is clear that extensive bronchiolar obliteration may occur in the distal divisions of bronchi or bronchioles which originally supplied the emphysematous spaces which now surround them. The commonest explanation for the dilatation of bronchi and bronchioles is that requiring collapse of the lung and inspiratory traction on the wall of the passage (Warner and Graham, 1933; Andrus, 1940; Coope, 1948; Fleischner, 1949). However it is obvious that these passages can dilate even in the presence of increased pressure in the surrounding lung. The only acceptable explanation in such circumstances is that the bronchi or bronchioles dilate because of the pressure of retained secretions, particularly in inflammatory episodes, which dilate the passage, such dilatation becoming permanent with destruction of the elastic mural elements. This is consistent with the views of Whitwell (1952), in which he distinguishes "atelectatic" bronchiectasis from the much commoner type in which lung collapse does not occur at any stage of its evolution.

ACKNOWLEDGEMENTS

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A CASE OF AMAUROTIC FAMILY IDIOCY WITH LIPID STORAGE DISEASE OF BONE[1]

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SUMMARY

A case of amaurotic family idiocy is described in which lipid storage disease of bone marrow closely resembling Gaucher's disease developed. The bone changes became evident at the age of twenty-one years, thirteen years after the neurological condition had been recognized.

The literature relevant to the case is reviewed and its significance discussed.

SINCE the term amaurotic family idiocy was coined by Sachs (1896) as a clinical description of a disease of infants characterized by dementia and blindness associated with cherry-red spots at the maculæ, its meaning has undergone considerable expansion. Now included are all those cases in which distension of ganglion cells with lipid material is found throughout the nervous system on histological examination. When the disease presents in late infancy or childhood, some of the cardinal signs may be absent. Thus macula changes are usually lacking, blindness or dementia may be absent, and sporadic cases are not infrequent (Wilson, 1954). The term is therefore inexact as a description of many cases of this disease, as it is in the one to be described.

The relation of amaurotic family idiocy to the generalized forms of lipid storage disease, especially Niemann-Pick and Gaucher's diseases, has long been a subject for speculation. It is generally regarded as a metabolic defect confined to nervous tissue, and no report has been found of its association with lipid storage disease of other organs.

A patient suffering from amaurotic family idiocy is described who survived into adult life and developed lipid storage disease of bone marrow resembling Gaucher's disease.

REPORT OF CASE.

The patient was a young woman, aged 21 years. Little was known of her first year, but she was said to have been normal at birth. At the age of a year, she was unable to sit unassisted. She made no attempt to walk until she was three and never learned to walk steadily because of sudden unexplained falls. She began school at the age of five, and at the age of eight was found to have defective vision. She was admitted at this time to the National Hospital under Dr. Gordon Holmes and was found to have the retinal changes typical of Tay-Sachs disease, but with no evidence of intellectual deterioration. From the age of ten she became increasingly disabled by involuntary jerkings of the limbs which, together with weakness of the legs, caused her to become bedridden at the age of fourteen. In the next seven years the weakness and jerking of her limbs gradually increased, and her sight slowly deteriorated, but there was no evidence of more than slight decline in intellectual powers.

Family History (Figure 1)

There was no Jewish blood or consanguinity in the family.

The patient's brother, aged 31 years, was well and had two healthy children. Her sister, who was normal at birth, died at nine months of " marasmus ". She was said to have become blind before death.

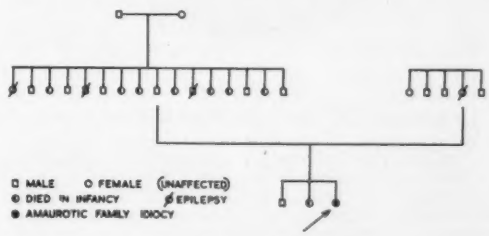

FIGURE 1
Family tree

The patient's mother was an aggressive epileptic, whose fits began at the age of 21 years. Her sight was normal. She had four siblings, who were well and who had numerous children and grandchildren. No other member of this family was known to have had fits, nor was there any history of death in infancy.

The patient's father was one of 16 siblings. The eight males were alive and well, except that two suffered from epilepsy. The eldest female was alive and aged 72 years, and also was subject to fits. The seven other females died between the ages of six and eighteen months. They were said to have been normal at birth, but to have gone blind and withered away. The surviving males had a large number of children and

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grandchildren. As far as could be discovered, none of these children had died in infancy, nor did any have evidence of neurological disease.

The patient's paternal grandparents died in old age, one of cancer and one of pneumonia.

Clinical Examination

The patient was observant with an accurate memory and a lively sense of humour. Allowing for defective eyesight and lack of schooling her I.Q. was 95.

The peripheral visual fields were full to confrontation with large central scotomata. At the macula of each eye was a red spot surrounded by grey discoloration. The optic disks were pale (Figure II). Eye movements were full, with a constant pendular nystagmoid movement constant in all directions of gaze.

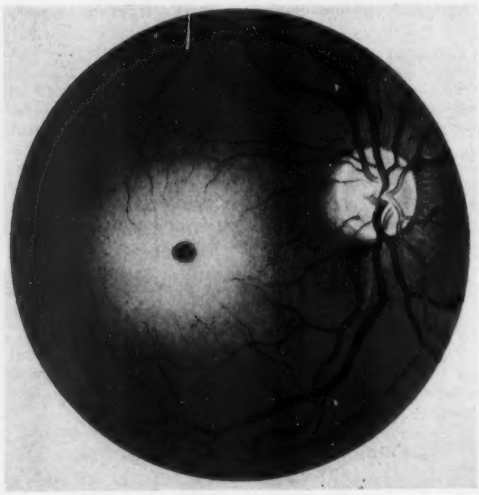

FIGURE II
A drawing of the optic fundus

The limb muscles were thin, and there was generalized weakness most severe in the legs. Tone was slightly increased in the limbs. Tendon reflexes were present, and the plantar responses were absent. Sensation was normal. Almost constant myolonic jerks involved the muscles of the limbs, trunk and face. These were aggravated by voluntary movement and were readily evoked by cutaneous stimuli or by eliciting tendon reflexes.

General bodily development was small, but menstruation and secondary sexual characteristics were normal. The liver and spleen were not palpable, and there was no enlargement of lymph glands. There was no pigmentation of the skin or pingueculæ. The cardio-vascular and respiratory systems were normal.

Investigations

Examination of the peripheral blood showed a hæmoglobin value of 87%. The red blood cells showed mild anisocytosis. The white cell count was 6000 per cubic millimetre, made up of polymorphs 56%, lymphocytes 36% and monocytes 8%. The platelets numbered 267,000 per cubic millimetre.

The total serum cholesterol content was 250 milligrammes per 100 millilitres.

Radiological examination of the skull and chest showed no abnormality.

An electroencephalogram was reported on by Dr. W. A. Cobb. Alpha rhythm was scarcely recognizable. Low voltage fast activity was present in all areas with scattered low amplitude waves at five to seven cycles per second. Frequent high voltage waves associated with scattered spikes occurred, the larger of which were associated with a jerk of the limbs.

FIGURE III
Spontaneous fracture of the upper shaft of the femur. Cystic changes in the bone are seen above and below the fracture site

The case was considered to be one of amaurotic family idiocy.

Some months later the patient complained of pain in the left leg and shortly afterwards suffered a spontaneous fracture of the upper end of the shaft of the femur. X-ray examination showed the presence of cystic changes in the bone in the region of the fracture (Figure III), and examination of other long bones revealed widespread changes. There was lack of trabeculation with marked thinning of the cortical bone, particularly noticeable in the tibiæ and the

lower ends of the femora. In the humeri and upper ends of the radii the inner margin of the cortex was scalloped, suggesting replacement of bone marrow with other tissue (Figure IV).

FIGURE IV

Lower end of the humerus showing scalloping of the inner margin of the cortical bone

The sternal bone marrow was very cellular and showed a moderate hyperplasia of normoblastic erythropoiesis. Scattered among the marrow cells was a small number of large rounded cells with a foamy cytoplasm which was stained pink by the periodic acid-Schiff method (Figure V).

DISCUSSION.

The diagnosis of amaurotic family idiocy was made on the grounds of the characteristic retinal changes, the evidence of widespread neurological disease with myoclonic jerking and the family history of blindness and death in infancy. Unusual features of the case before disease of bone supervened were the absence of gross dementia and the survival into adult life of a patient with the retinal changes of the infantile form of the disease.

The radiological features of the bony changes were similar to those seen in Gaucher's disease. Thinning of the cortex and expansion at the ends of long bones, especially the femur, are characteristic. Spontaneous fracture may occasionally occur, and cystic changes, when present, are usually found, as in this case, at the upper end of the femur (Thannhauser, 1950). The scalloping of the inner margin of the cortex in long bones in the arm suggested that bone marrow replacement was widespread.

Evidence as to the nature of this replacement was provided by the presence in the sternal bone marrow of cells morphologically similar to the foam cells characteristic of Gaucher's disease. The staining reaction to the periodic acid-Schiff method of the contents of these cells was compatible with a cerebroside, though positive identification as such cannot be made on these grounds alone.

It is generally accepted that the lipid changes in amaurotic family idiocy are confined to the nervous system. The majority of pathological reports describe only these changes; but where a careful search has been made, evidence of lipid disease elsewhere has not been found (Sachs, 1929; Schlesinger and Greenfield, 1934; Rothstein and Welt, 1941; Globus, 1942; Ford, 1952). An exception is a case described by Davison (1942), who in a typical example of Tay-Sachs disease found foam cells in the liver and spleen. These organs were not enlarged.

On the other hand, there are a number of reports of neurological manifestations associated with generalized lipid storage disease in infants. In Niemann-Pick disease dementia and paralysis may occur (Rothstein and Welt, 1941), and blindness with macula changes resembling those seen in Tay-Sachs disease may be found (Goldstein and Wexler, 1931). In Gaucher's disease in infants a striking neurological syndrome, consisting of dementia, convulsions and strabismus with an attitude of opisthotonus

and generalized rigidity, has been described (Seitz and Stammler, 1951 ; Debre *et alii*, 1951 ; Aballi *et alii*, 1938 ; Meyer, 1934 ; Oberling and Woringer, 1927). Hepatosplenomegaly accompanies the development of these symptoms,

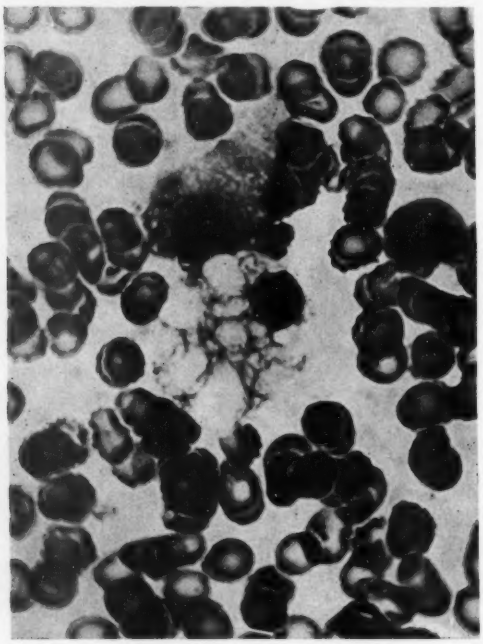

FIGURE Va

Sternal bone marrow. Leishman stain. Showing a foam cell. ×900

and death usually occurs before the end of the first year. There is some variation in the pathological findings reported in the nervous system in these cases. The cortical nerve cells are described as shrunken and dense by Oberling and Woringer (1927), while Schairer (1948) and Seitz and Stammler (1951) found swollen and vacuolated cells in the basal ganglia in addition to atrophic changes and glial proliferation in the cortex. Debre *et alii* (1951) found Gaucher cells in the perivascular spaces of the brain. These authors are agreed, however, that the changes found can readily be distinguished from Tay-Sachs disease.

Disease of the nervous system in association with Gaucher's disease in later life is rare, and in a review of 89 cases Hoffman and Makler (1929) found no report of changes in the central nervous systems in patients over one year of age. Van Bogaert and Froehlich (1939) described the development of rigidity of extrapyramidal type in the legs of a forty-two-year-old man who had suffered from Gaucher's disease since the age of twelve. This occurred in the terminal stages of his illness, and no post-mortem examination was made. Davison (1929) observed degenerative changes in the basal ganglia of a twenty-six-year-old man suffering from Gaucher's disease. He did not regard them as being directly associated with lipid disease, but ascribed them to chronic liver disease. Two reports concern children. Reiss and Kato (1932) described the occurrence of hepatosplenomegaly in three of six children of healthy Japanese parents. In two a splenectomy was performed, and the characteristic changes of Gaucher's disease were found. In their sixth year these two

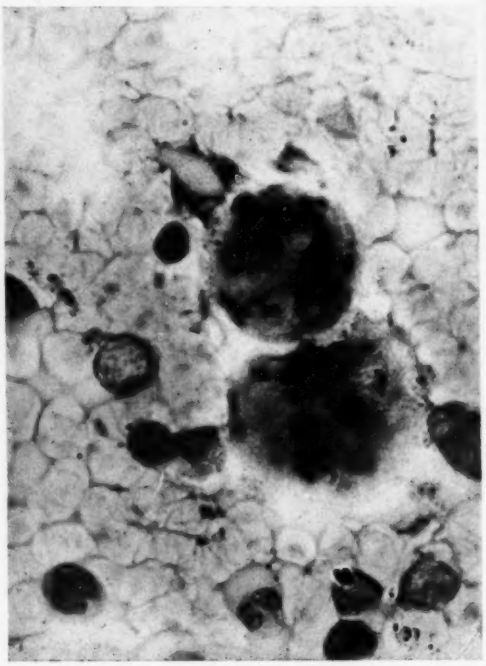

FIGURE Vb

Sternal bone marrow. PAS stain. Showing PAS-positive contents of foam cells. ×1000

children developed dementia, failing vision with pale optic disks, strabismus and progressive spasticity of the limbs with continuous clonic jerkings, the illness terminating fatally in both cases about a year after the onset. The third child died at the age of two years. The nervous system was not examined, but hepato-

splenomegaly was present. No post-mortem examinations were made. The case of a boy of eleven years with Gaucher's disease is fully described by Bird (1948). Development was normal until the age of seven, when progressive intellectual deterioration began, followed by *petit mal* seizures. In the last year of his life he was severely demented, with "lead pipe" rigidity and exaggerated reflexes in his limbs, but normal vision and optic fundi. Histological examination of the brain revealed widespread ballooning of nerve cells, with Gaucher cells in the liver, spleen and lymph glands. Bird considered the changes in the nervous system to be strikingly similar to those found in three cases of amaurotic family idiocy reported in the same paper.

No further reports of involvement of the nervous system in the adult form of Gaucher's disease were found. The combination is clearly rare, and in each of the above cases, except perhaps the last, neurological disease appeared in the context of established hepato-splenomegaly. No case similar to the one here described could be found in which involvement of other organs in lipid storage appeared in established amaurotic family idiocy.

Although there is no detailed pathological or biochemical proof, it is considered that the clinical features of this case make a diagnosis of amaurotic family idiocy reasonably certain, and that the radiological and histological findings in bone and bone marrow closely resemble Gaucher's disease. It is unlikely that the changes in the nervous system and those in the bone marrow are unrelated, both presumably being evidence of an inborn error of lipid metabolism. The question arises whether this combination is unique in this case, or whether it is a possibility in other patients suffering from amaurotic family idiocy who survive long enough for a slow accumulation of lipid material to become evident in other parts of the body. It is possible that the fate of the patient's sister and paternal aunts represents the usual course of the disease, the epilepsy of other members of the family on the paternal side its partial expression and the patient its potentialities. At least this case, together with those quoted above, suggests a fundamental link between the cerebral lipid changes in amaurotic family idiocy and generalized lipid storage disease.

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THE NATURE OF LEUCHÆMIA

I. A HISTOCHEMICAL STUDY OF THE LEUCHÆMIC CELL IN MAN[1]

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SUMMARY

A component of the globulin fraction of rabbit serum reacts with the protoplasmic constituents of the normal cell but not with the corresponding malignant counterpart. The phenomenon can be demonstrated by conjugating a serum globulin fraction with fluorescein isocyanate; non-neoplastic tissues stain, but the neoplastic cells do not. This has been established particularly with experimentally produced hepatomata in rats and has been confirmed with other experimentally produced tumours.

The method has been applied to naturally occurring tumours in man, and similar results have been obtained; that is to say, normal tissues stained with the fluorescein isocyanate-globulin complex fluoresce brightly, but tumour tissues do not.

Films obtained from normal individuals, as well as those suffering from infectious conditions such as glandular fever, were compared, using this technique, with those of leuchæmias. Normal white cells and those from various infectious diseases fluoresced well. Among the leuchæmias, the cells of chronic myeloid, chronic lymphatic and monocytic leuchæmias all fluoresced brightly. A proportion of the cells of acute leuchæmias (the primitive cells) failed to fluoresce.

This observation suggests that the chronic leuchæmias are non-neoplastic conditions but that the acute leuchæmias, from the point of view of this method of study, do conform with the postulate that they are neoplastic in nature.

RESEARCH in recent years has elucidated many questions which, only three decades ago, seemed unanswerable. This has been achieved partly by use of methods already employed but also by discovery of new techniques. Each advance has itself imposed new problems, and, though infections and metabolic diseases are now better understood, a wealth of accumulated discovery has still not made the problem of leuchæmia simpler. An observation bearing on this topic is presented here.

HISTORICAL NOTE

Early in the century Banti (1904), Ziegler (1906) and Ribbert (1907) regarded the leuchæmias as neoplasms, and the authority of their names doubtless influenced acceptance of this view. However, immediately this opinion was questioned (Veszprémi, 1906; Pappenheim, 1907; Fabian, Naegeli and Schatiloff, 1907; Kaufmann, 1911). For a quarter of a century various writers have placed the leuchæmias variously amongst infections, neoplasms and diseases of unknown origin. In 1914, Mallory propounded the view that lymphatic leuchæmia, lymphosarcoma and Hodgkin's disease were variants of one kind of tumour. Warthin (1931) reviewed the subject and grouped the leuchæmias with the lymphatic tumours, and this view has been accepted especially by authors interested in neoplastic disease (Ewing, 1928; Willis, 1947).

The main reasons for the neoplastic view of leuchæmia are the gross and atypical hyperplasia with infiltration of the tissues (often with tumour-like growths), the occurrence of the disease after the action of certain chemical agents which also produce other tumours in man (Mallory, Gall and Brickley, 1939), the similarity of the metabolism of the cells to those of tumours (Isaacs and Arbor, 1932) and the fatal nature of the disease. The most cogent arguments against this hypothesis deal with cell peculiarities. Unlike the true tumours, whose cells maintain their original character, in-vitro cultures of leuchæmic cells show a differentiation to normal (Israëls, 1940). When grown with tubercle bacilli (Timofejewsky and Benewolenskaja, 1927) they differentiate in a way not seen with tumour cells. Furthermore, they do not grow in the anterior chamber of the eye of the guinea-pig (Hoogstratten, 1949) as demonstrated with other tumours by Greene (1948).

[1] Received on June 20, 1957.
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The evidence has been reviewed by various writers (Englebreth-Holm, 1941 ; Whitby, 1956), and it is clear that the matter is still by no means settled.

In the last few years evidence has been accumulating which shows that the malignant cell differs from the normal prototype in that it lacks a protoplasmic component present in the normal cell. This idea originated in the work of Miller and Miller (1947), who showed that when rats were fed with a chemical carcinogen — 4-dimethylaminoazobenzene — the dye combined with a protoplasmic constituent, one of the soluble proteins of the cytoplasm. It was shown that when tumours developed, the cells of the tumour lacked this component. Considerable work has been done in this direction, and electrophoretic observations show the particular component to be one of the basic proteins of the cytoplasm.

Weiler (1952, 1956*a*, *b* and *c*) investigated this question by serological and histo-serological methods. He found that " organ specific antibodies " to rat liver, produced in rabbits and conjugated with fluorescein isocyanate, would stain the cells of normal liver so that they fluoresced brightly in ultraviolet light ; the hepatoma cells did not stain, however, and thus tumour tissue could be readily differentiated from the normal.

Subsequent investigators (Hughes *et alii*, 1957) have shown that this effect can be produced with fluorescent normal rabbit serum and have concluded that the phenomenon is a more general one than merely an antigen-antibody reaction. Further still Louis (1957*b*) has shown that a cell actually in the process of mitosis stains with fluorecent rabbit globulin ; so that the loss of staining is not a function of cell division but of the neoplastic state itself.

Whatever the fundamental significance of this phenomenon, it seems clear that there are chemical differences between the malignant and the non-malignant cell which can be readily demonstrated in the tissues of the experimental animal. Also, experiments on several human tumours which are being carried out in this department indicate that it is a general property of neoplastic tissue not to stain with fluorescent globulin, whereas normal tissues do.

It seemed desirable and appropriate, therefore, that this method should be applied to examples of the various forms of leuchæmia.

METHOD AND MATERIALS

White blood cells were prepared from fresh human blood by slow centrifugation. After pipetting off the buffy coat, frequent washing and centrifugation with saline were carried out until a specimen of white blood cells of adequate homogeneity was obtained. All blood used was taken from donors of group A, Rh-negative. The white cells were suspended in 0·15 M. saline to give a 25% suspension and then injected intramuscularly into adult rabbits three times per week. The initial dose of 0·5 cubic centimetre was gradually increased to 2·0 cubic centimetres over a period of three weeks.

After a further two weeks the rabbits were bled, and complement fixation tests were performed on the antisera, using a 25% suspension of white blood cells as an antigen and two minimum hæmolytic doses of fresh guinea-pig complement. Doubling dilutions of antiserum were used. For the indicator system, hæmolysin[1]-sensitized washed sheep red cells were used. Generally, the sera showed titres of about 1 : 320 taking as the end-point that dilution giving 50% hæmolysis of the indicator system. From satisfactory sera a globulin fraction was prepared by precipitation with half-saturated ammonium sulphate, and, after dialysis, this was conjugated with fluorescein isocyanate prepared by the method of Coons and Kaplan (1950). After three absorptions of these antisera with fresh human liver particles, complement fixation tests were performed, and the titres were found to be about 1 : 80 using either liver or whole cells as antigens. Similar globulin fractions were prepared from normal rabbits and, to provide controls, were conjugated in a like manner.

Blood smears were taken on dry slides, which were then immersed in cold methyl alcohol (−70° C) in a Coplin jar for 15 to 30 minutes, and dried for one and a half hours in a cold room at 0–2° C with the help of a fan, after which they were ready for staining.

Prior to staining, the unbound fluorescein and its derivatives were extracted with ethyl acetate (Dineen and Ada, 1957). The slides were then stained with the fluorescent antiserum for 15 minutes at room temperature, washed in three changes of buffered saline, pH 7·3, for 10 minutes and then examined with a Leitz fluorescence microscope. Smears from healthy people with normal blood findings were treated and examined in the same way for comparison.

The source of light for ultraviolet microscopy was a 10-ampère D.C. carbon arc lamp with clockwork feed, a hardglass illuminating lens, and a filter cooling cell. The filters were set in swing-out holders carrying two filters 2-milli-

[1] Commonwealth Serum Laboratories.

metre UG 1, two filters 4-millimetre BG 12, one green filter and one light subduing filter. The microscope was fitted with special apochromatic fluorescence objectives with integral Euphos glass filters, and slides were used without cover glasses. The eyepieces were of periplanatic type, fitted with an ultraviolet protection filter 2·5-millimetre (Euphos) and a Wratten G15 gelatin filter.

For photography "Isopan F" film (Agfa) was used with exposure times of one and a half minutes. After being photographed the smears were stained by Leishman's method, and the same fields were again photographed with visible light.

RESULTS

The staining characteristics of normal circulating white blood cells were investigated in 12 subjects, whose ages ranged from 10 years to 60 years and who had normal blood films. Smears from each of these people were stained with: (i) fluorescein—anti-human leucocyte rabbit globulin complex; (ii) fluorescein—normal rabbit globulin complex.

The general finding was that the red blood corpuscles failed to fluoresce, whilst each individual type of white blood corpuscle fluoresced well in ultraviolet light. Only the cytoplasm of these cells fluoresced and, in the granular leucocytes, the fluorescence appeared to be mainly confined to the cytoplasmic granules.

Marrow smears were taken from three hospital patients under investigation for secondary carcinomatous deposits in bone. These were also stained with both sera, and it was found that all the "blast" cells of the myeloid, lymphocytic and monocytic series fluoresced. Secondary deposits of malignancy were not seen in any of these smears.

TABLE I

Clinical and Hæmatological Types of Leuchæmia Encountered in the Series of Cases Studied

Types of Cases	Number of Cases
Acute leuchæmia	14
Chronic lymphatic leuchæmia	46
Chronic myeloid leuchæmia	38
Monocytic leuchæmia	2
Total	100

Finally, atypical leucocytes (Figure VI*a*) and lymphocytes (Figure VI*b*) were investigated. These were taken from six cases of infectious mononucleosis in which the white cell count was raised, the blood film was typical and the result of the Paul-Bunnell test was positive for heterophil antibodies. In all these cases the cytoplasm of all the white blood corpuscles, as well as that of the atypical cells, fluoresced brightly and evenly.

TABLE II

Clinical Features and Hæmatological Characters (of the Peripheral Blood) in 14 Cases of Acute Leuchæmia[1]

Case Number	Sex	Type of Case	Age (Years)	Duration of Illness	Transfusion	Hæmoglobin Value (Percentage)	White Cells per Cubic Millimetre	Percentage of "Blasts"	Percentage of Cells Fluorescing
1	M.	Acute lymphatic	3½	3 weeks	—	55	124,000	82	40
2	M.	Acute lymphatic	40	8 years	T	60	3,500	72	90
3	M.	Acute myeloid	2	2 months	—	57	150,000	26·5	30
4	M.	Acute myeloid	68	15 years	T	60	30,000	22	95
5	M.	Acute lymphatic	15	2 years	T	63	65,000	50	75
6	M.	Acute lymphatic	8	1½ years	T	60	32,000	50	80
7	F.	Acute myeloid	46	4 years	—	40	80,000	47	60
8	F.	Acute myeloid	51	6 years	T	55	60,000	38	80
9	M.	Acute lymphatic	40	4 years	T	61	3,500	72	35
10	F.	Acute myeloid	22	6 months	T	70	46,000	58	50
11	M.	Acute myeloid	53	4 months	—	83	6,600	64	39
12	M.	Acute lymphatic	14	4 months	—	78	30,000	52	44
13	F.	Acute lymphatic	28	8 months	—	76	36,000	28	50
14	M.	Acute lymphatic	3½	2 months	T	58	3,800	70	18

[1] The proportion of cells showing fluorescence is given; it is noteworthy that the proportion is high, especially in those cases in which a recent transfusion has been given. Six of the cases arose as complications of chronic leuchæmias.

One hundred cases of leuchæmia (Table I) were studied. These were divided into the usual groups.

A. Acute Leuchæmias (*Figures I–III*).—In all, 14 cases were investigated; of these, eight were of acute lymphatic leuchæmia and the remaining six were of the acute myeloid type (Table II). Only the peripheral blood was examined by the present method. The general finding in these acute cases was that the more mature cell types fluoresced, while the more primitive ones failed to fluoresce in ultraviolet light. The number of non-fluorescing leucocytes appeared to depend on the percentage of "blast" cells in the peripheral blood, but clear-cut observations were prevented by the event of a transfusion, which greatly increased the number of fluorescing cells in the peripheral blood. Six of the cases occurred as an exacerbation of a chronic leuchæmia.

TABLE IIIA

Cases of Chronic Lymphatic Leuchæmia Divided into Four Arbitrary Groups According to the Blood Count at the Time of Examinations[1]

Group	Type of Case	Number of Cases
I	High white cell count: 100,000 cells per cubic millimetre	12
II	Moderate white cell count: 50,000 to 100,000 cells per cubic millimetre ..	2
III	Low white cell count: 5000 to 50,000 cells per cubic millimetre	28
IV	Aleuchæmic: 5000 cells per cubic millimetre	4

[1] This division is made solely as a simple means of presenting this aspect of all the cases and to emphasize that all forms were included in the series.

TABLE IIIB

Chronic Lymphatic Leuchæmia: Four Individual White Cell Counts, One from Each of the Groups Given in Table IIIA

Group	Typical Differential Count in Each Group			
	Lymphocytes *per Centum*	Lymphoblasts *per Centum*	Neutrophils *per Centum*	Smudges *per Centum*
I	74	2	20	4
II	81	4	12	3
III	61	1	15	3
IV	80	2	18	0

The photomicrographs representing the acute lymphatic type (Figures I and II) illustrate the case (Case I) of a boy, aged three and a half years, who was perfectly well until three days prior his admission into hospital. The child became listless and anorexic and developed swollen glands in the cervical, axillary and inguinal regions. Small petechiæ appeared on both thighs and arms at first and later on the abdomen, thorax, eyelids, gums and soft palate. The liver was palpable seven centimetres below the costal margin, and the spleen reached well below the level of the umbilicus. Despite blood transfusions the child died 10 days after his admission into hospital. At post-mortem examination performed one and a half hours after death, sections were taken from the liver, spleen and lymph nodes. All the cells in the lymphocytic infiltrations in these organs appeared to fluoresce (Figure II).

B. Chronic Lymphatic Leuchæmia.—Peripheral blood smears from 46 in-patient and out-patient subjects were investigated. All the white blood corpuscles that were examined in each of the cases fluoresced (Figure IV). The cases may be divided arbitrarily into four groups depending on the number of white cells found. These are: (*a*) high white cell counts, in which the count is greater than 100,000 cells per cubic millimetre; (*b*) moderate white cell counts (ranging from 50,000 to 100,000 cells per cubic millimetre); (*c*) low white cell counts (ranging from 5000 to 50,000 cells per cubic millimetre); and (*d*) an aleuchæmic group with a count of less than 5000 cells per cubic millimetre. This division has been made solely with the object of presenting the range of the white cell counts of the cases in simple form. The number of cases in each group (Table IIIA) and, in addition, typical examples of the differential white cell count (Table IIIB) are given.

C. Chronic Myeloid Leuchæmia.—In this group 38 cases were investigated; again all the white blood corpuscles that were examined fluoresced (Figure V). These cases also are presented in a similar form to those of the chronic lymphatic group (Tables IVA and B).

Only two cases of monocytic leuchæmia were examined, and in both the fluorescence was complete and uniform.

Although in most cases only the white cells showed a positive staining reaction, in four of the cases the red cells also fluoresced more or less brightly. This was found to occur in cases with a positive response to the Coombs test and appeared to be an expression of the association (in this small proportion of cases) of hæmolytic anæmia with leuchæmia (Figure VII).

In order to test the proposition that a clear distinction can be made between malignant

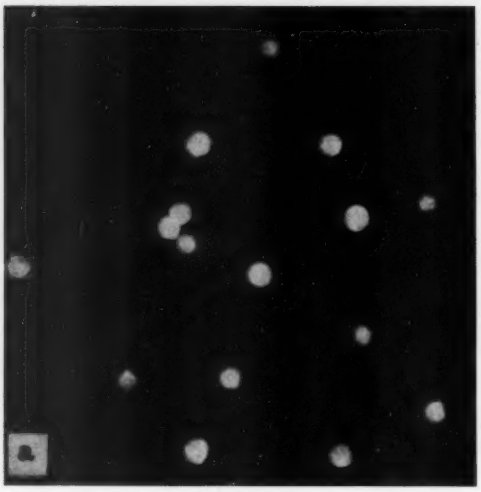

FIGURE IA

Photomicrograph of blood film taken from a patient suffering from acute lymphatic leuchæmia, stained with fluorescein-globulin complex and photographed on ultra-violet light, showing fluorescing white cells. (×450)

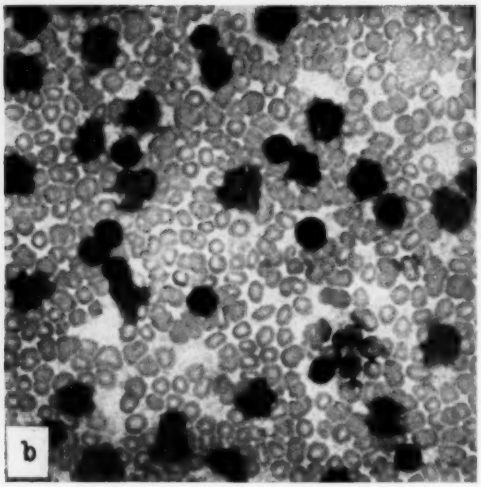

FIGURE IB

Photomicrograph of blood film taken from a patient suffering from acute lymphatic leuchæmia, showing the same area as Figure IA subsequently stained by Leishman's method—only 40% of white cells fluoresce (compare with Figure IA)). The non-staining cells were the immature types; these appeared to be susceptible to damage by ultra-violet and blue light. (×450)

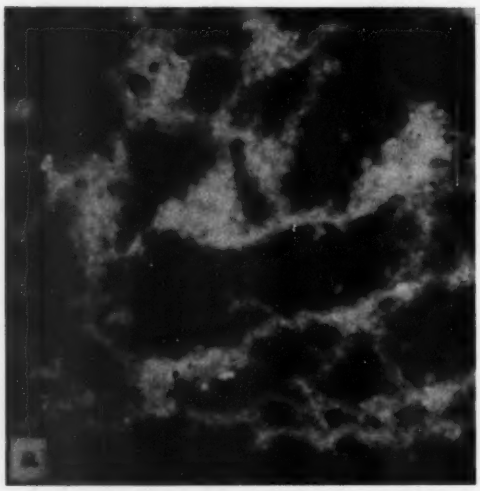

FIGURE IIA.

Leuchæmic lymph node from same patient as Figure I. Unfixed frozen section stained with fluorescein-globulin complex showing, with ultra-violet light, fluorescing lymphocytes. (×240)

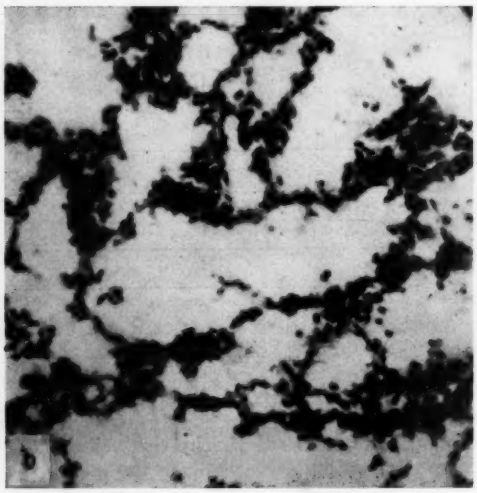

FIGURE IIB

Leuchæmic lymph node from same patient as Figure I. Same area as Figure IIA subsequently fixed with formalin and stained with hæmatoxylin and eosin for comparison with Figure IIA. (×240)

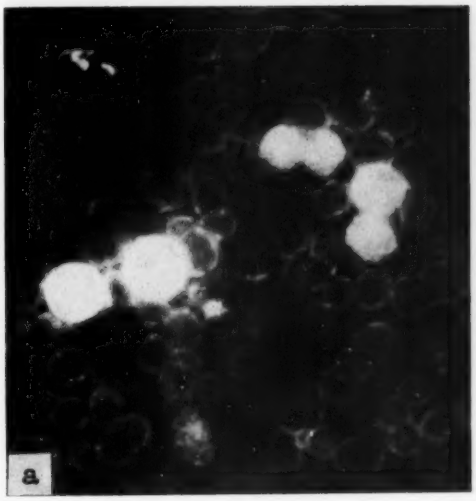

FIGURE IIIA

Photomicrograph of blood film taken from a patient suffering from acute myeloid leuchæmia, stained with fluorescein-globulin complex showing, with ultra-violet light, fluorescing white cells. Weak fluorescence of erythrocytes is also seen; this is possibly associated with the positive response to the Coombs test present. (×750)

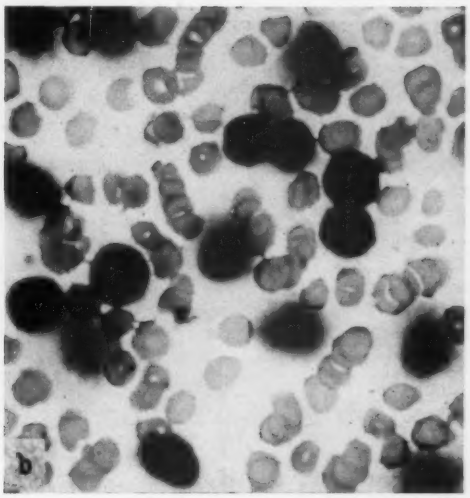

FIGURE IIIB

Photomicrograph of blood film taken from a patient suffering from acute myeloid leuchæmia, same area as Figure IIIA, subsequently fixed and stained by Leishman's method to show that some white cells did not fluoresce (compare with Figure IIIA)). Damage to cells is evident; this is due to ultra-violet light and appears more obvious with longer exposures. (×750)

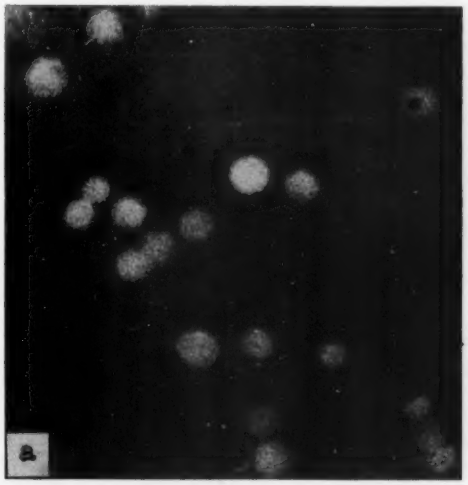

FIGURE IVA

Photomicrograph of blood film taken from a patient suffering from chronic lymphatic leuchæmia, stained with fluorescein-globulin complex and showing, with ultra-violet light, fluorescing white cells. (×650)

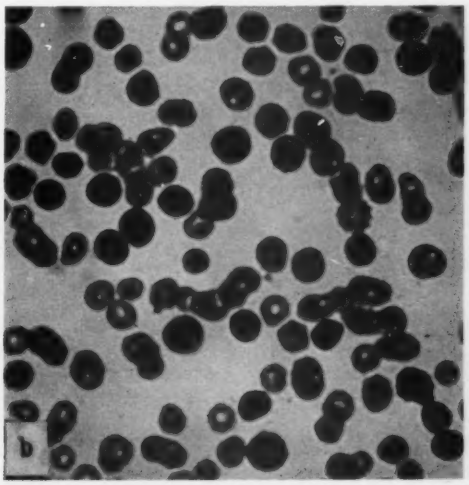

FIGURE IVB

Photomicrograph of blood film taken from a patient suffering from chronic lymphatic leuchæmia showing same area as Figure IVB after staining by Leishman's method to show that all white cells fluoresced (compare with Figure IVA). (×650)

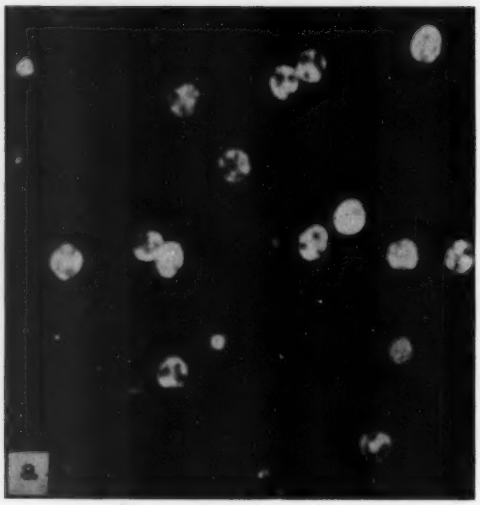

FIGURE VA

Photomicrograph of blood film taken from a patient suffering from chronic myeloid leuchæmia, stained with fluorescein-globulin complex and showing, with ultra-violet light, fluorescing white cells. (×450)

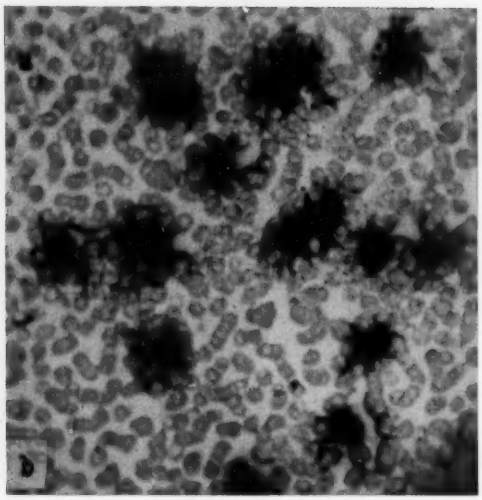

FIGURE VB

Photomicrograph of blood film taken from a patient suffering from chronic myeloid leuchæmia showing the same area as Figure VA subsequently stained by Leishman's method to show that all the white cells fluoresced (compare with Figure VA). (×450)

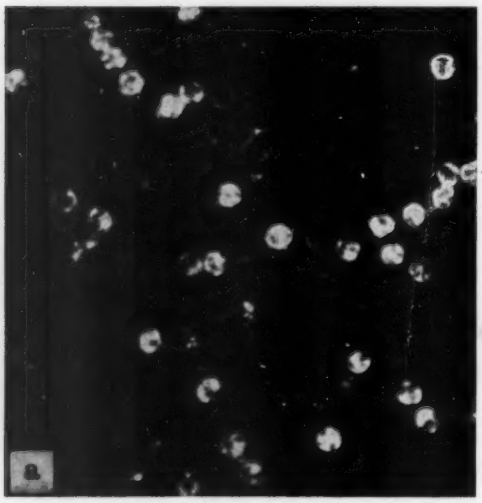

FIGURE VIA

Fluorescence photomicrograph from a case of leucocytosis showing fluorescing polymorphonuclear leucocytes. (×450)

FIGURE VIB

Fluorescence photomicrograph from a case of infectious mononucleosis showing fluorescing atypical lymphocytes. (×450)

and normal cells by the fluorescein-globulin conjugate staining method, extensive studies were made on malignant tumours. The original observations on liver tumours in rats, following feeding with 4-dimethylaminoazobenzene, were repeatedly confirmed. Numerous examinations have been made on liver tumours due to administration in the food of many other dyes; these have all shown the same result. This work will be recorded elsewhere.

TABLE IVA

Chronic Myelogenous Leuchæmia: The Four Arbitrary Groups are Given here as with the Lymphatic Leuchæmias

Group	Types of Cases	Number of Cases
I	High white cell count: 100,000 cells per cubic millimetre	10
II	Moderate white cell count: 50,000 to 100,000 cells per cubic millimetre ..	6
III	Low white cell count: 5000 to 50,000 cells per cubic millimetre	18
IV	Aleuchæmic: 5000 cells per cubic millimetre	4

TABLE IVB

Chronic Myelogenous Leuchæmia: Four Individual White Cell Counts, One from Each of the Groups, Given in Table IVA

Group	Typical Differential Count in Each Group				
	Neutrophils *per Centum*	Metamyelocytes *per Centum*	Myelocytes *per Centum*	Basophils *per Centum*	Lymphocytes *per Centum*
I	68	18	10	2	2
II	61	22	12	3	2
III	64	11	1	3	17
IV	70	10	14	0	6

Observations were made also on a group of malignant tumours in man; these were obtained principally from the operating theatre but some were procured *post mortem*.

Thus, 30 solid tumours of various organs were investigated by this method of study (Table V). Unfixed frozen sections from each of these tumours were cut by a method already described (Louis, 1957*a*) and stained both with the specific fluorescent antiserum and with the normal fluorescent serum. Differential staining was observed in all cases using both sera; the normal cells fluoresced, whereas the malignant counterparts did not.

DISCUSSION

Although a great deal has been written about the leuchæmias, much of this is speculation based on inadequate information. The factors responsible for incomplete maturation of the leucocytes and the appearance of these immature cells in the peripheral blood are of fundamental biological importance, but relatively little attention has been paid to them.

Since so much attention has been given to the hypothesis that these conditions are neoplasms, this method of study, which has been used recently with other tumours (Table V), has been applied to the leuchæmias. The staining of normal tissues of various kinds and in various animals has been demonstrated sufficiently often for this to be accepted as a satisfactory control.

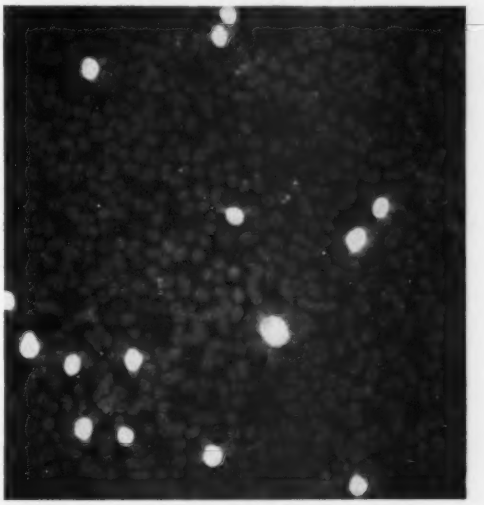

FIGURE VII

Fluorescence photomicrograph of blood film taken from a case of chronic lymphatic leuchæmia with a positive response to the Coombs test. The white cells show a brighter fluorescence than the red cells. (×320)

The evidence for the specificity of this fluorescence staining method as a means of distinguishing between normal and neoplastic tissue has depended almost solely on the experimental work on hepatomata induced in rats by feeding with 4-dimethylaminoazobenzene. This staining method was extended, during the present investigation, to a study of 30 tumours in man. Though the matter requires further scrutiny, the uniformly negative results obtained with these growths, as compared with the

uniformly positive staining of the normal tissues, seem a clear indication that non-neoplastic cells in post-natal life always stain and that neoplastic cells do not. Whether primitive embryonic cells stain has not yet been determined.

One feature of importance is that the indubitable neoplasms of the skin show the characteristic features. The observations of Hiramoto and Pressman (1957) that squamous and melanotic growths do not share these characters raise the question of the criteria for diagnosis of carcinoma, as opposed to *molluscum pseudo-carcinomatosum*, and melanoma, as opposed to non-neoplastic moles. Personal observations have all indicated that normal tissues stain positively and that neoplastic tissues do not.

TABLE V

The Various Malignant Tumours Examined by the Fluorescin Isocyanate-Globulin Staining Technique[1]

Tumour	Number of Cases
Breast:	
Carcinoma	4
Skin:	
Squamous carcinoma	2
Basal-cell carcinoma	2
Lung:	
Carcinoma	4
Stomach:	
Carcinoma	1
Liver:	
Hepatoma	3
Caecum:	
Carcinoma	3
Colon:	
Carcinoma	7
Rectum:	
Carcinoma	3
Lymph node:	
Reticulosarcoma	1
Total	30

[1] In all cases staining was carried out with the specific antiserum conjugate as well as (in control sections) with a normal serum conjugate. In all cases, though the normal tissue fluoresced brightly, the tumour tissues failed to stain.

The examination of this series of cases of leuchæmia by the fluorescence staining method shows that many of the white cells (and in some cases all of them) stain well. These observations emphasize several features: first, leuchæmia is neither a single condition nor even a homogeneous group; secondly, the presence of the auto-antibodies which result in the staining of all the blood cells in some cases could conceivably give false positives; thirdly, the relation of the non-staining cells to primitive and embryonic bone marrow cells requires study.

The cases investigated fall naturally into two groups: that in which all the white cells fluoresce (86 cases) and that in which only some do so (14 cases). In the former group the cases belonged, clinically, to the chronic myeloid and lymphatic leuchæmias; whereas those of the second type, in which only some cells fluoresce, were, clinically, acute leuchæmias. The examples of monocytic leuchæmia examined belonged to the first group and showed universal fluorescence.

In some cases the red blood corpuscles were found to stain as well as the white cells (Figure VII). This is due, probably, to the presence of absorbed globulin and was found in individuals suffering from acquired hæmolytic anæmia. In this regard it is significant that a proportion (about 6%) of patients with leuchæmia suffer also from hæmolytic anæmia. This phenomenon is related to the positive response to the Coombs test and will be discussed in detail elsewhere. Though some normally non-staining white cells might theoretically show staining in these cases, the small number of these cannot significantly influence the series. In any case, the white cells in these cases fluoresce more strongly than in the cases in which red corpuscle fluorescence is not observed; so that the degree of intrinsic fluorescence is recognizable.

The nature of the non-staining cells is still not quite clear; the problem is whether they are merely dedifferentiated, that is "embryonic" in character, or are actually neoplastic. Examination of the cells showed that the non-staining forms were the least differentiated and that non-staining differentiated cells were not seen. These non-staining cells corresponded in some degree with the "blast" cells. Generally they were much fewer in number (Table II); but in so far as transfusions had been given recently in most cases, this discrepancy might be explained as a result of them.

White corpuscles in conditions such as glandular fever, even though numerous and atypical, stained in the same way as did normal cells. Study of cells from bone marrow punctures of individuals not suffering from leuchæmia was made, but non-staining cells were not found. This suggests that the non-staining cells in the peripheral blood are neoplastic; nevertheless, this cannot be maintained with complete certainty until more specimens of bone marrow have been examined, since blast cells normally occur so infrequently in marrow. The difficulties of ensuring that all cells stain, and that an occasional non-staining cell has not been overlooked, will be appreciated particularly by those who have used this method of study.

On the basis of these observations it seems clear that the conditions of chronic leuchæmia (myeloid and lymphatic) and monocytic leuchæmia are non-neoplastic diseases comparable with hyperplastic states in other organs. In this regard some of the hyperplasias of the reticulo-endothelial system exemplified by enlargement of lymph nodes and spleen are specially significant. Some of these, though often progressive and fatal, are not regarded as neoplasms; some are known to be infectious, and others are "metabolic" in character. On the other hand the acute leuchæmias do conform, as far as the present study is concerned, with the proposition that they may be neoplastic.

In leuchæmia there is the same kind of problem that there is in the histological diagnosis of many malignant tumours of solid tissues. The criteria are not apparent necessarily at the simple morphological level—it usually depends on behaviour: do they show the characteristics of persistent growth? In the blood the principal difference between some leuchæmic cells and those of hyperplastic bone marrow is largely numerical. The staining characteristics discussed here may be another difference, but this is not yet certain; cultural characters would suggest that differences are not significant.

Another possibly discordant feature in the acceptance of the neoplastic hypothesis is that only the "primitive" type cells do not stain. In most tissue neoplasms some of the cells show a change in the direction of differentiation; without this, histological classification would not be possible. All the tumours examined in this study showed this feature, sometimes in a gross form, and in these even the most differentiated cells failed to stain. It must be assumed that the cells in leuchæmia do not differentiate in the blood-stream, since non-staining differentiated cells were not seen; yet do so in tissue culture (Israëls, 1940).

More recently, Steinberg and Martin (1945) compared the antigenic distribution of normal and leuchæmic leucocytes using an agglutination method. They demonstrated four types of antigenic specificity corresponding to young granulocytes, adult granulocytes, young lymphocytes and adult lymphocytes. Their findings suggested that the antigenic specificity and chemical composition of the leuchæmic leucocytes were similar to those of normal leucocytes. Seligmann, Grabar and Bernard (1955), using the Ouchterlony method of precipitation in a gel, also were unable to demonstrate any differences between normal and leuchæmic cells. Thus the gross changes in the spectrum of the cytoplasmic proteins which occur in malignancy (Sorof and Cohen, 1951), which may be correlated with loss of fluorescence staining, are not a feature of the leuchæmias.

However, for the time being, the evidence suggests that the acute leuchæmias are neoplasms. In these cases, therefore, there must be two kinds of cells in a blood film; this is explicable on the assumption that non-neoplastic and neoplastic cells are intermingled. In the solid organs tumour cells are segregated from the normal tissue except at the sites of invasion, whereas in the fluid blood a more intimate mixing is to be expected.

One characteristic of leuchæmia, which has presented a difficulty in the easy acceptance of most hypotheses, has been the development of an acute leuchæmia as a complication in a chronic form. If the chronic forms be regarded as non-neoplastic hyperplasias, then we have the counterpart of a commonly observed phenomenon. The occurrence of a myosarcoma in a fibroid of the uterus, the development of a malignant sacro-coccygeal tumour in a sacral developmental abnormality and the frequent observations of a carcinoma in a polypus of the colon are all examples of malignancy in different kinds of non-neoplastic conditions.

An interesting feature of the controversy regarding the nature of leuchæmia is that the evidence collected from experimental work on animals, including naturally occurring leuchæmias of birds and rodents particularly, has pointed in the direction of a neoplastic nature (Engelbreth-Holm, 1941), whereas that obtained by a study of the disease in man has suggested the reverse (Whitby, 1956).

If we accept the proposition that leuchæmia is a symptom complex, having a diverse ætiology (a view which does not seem ever to have been seriously opposed) and showing more than one kind of pathological process, most of the difficulties disappear. It would seem that most of the forms seen in animals are neoplastic (though this question is being studied at present), but certainly most seen in man are not. At the same time there is a small group consisting of the acute leuchæmias, whether arising *de novo* or as a complication of a chronic type, which appear to be true neoplasms. The observations already made by Steinberg and Martin (1945) and by Seligmann, Grabar and Bernard (1955) on the immunological differences between the normal and leuchæmic cells are of significance here. It is obvious that future studies must take cognizance of the information obtained by these new methods of investigation.

ACKNOWLEDGEMENTS

My thanks are due to the many friends and colleagues, too numerous to mention individually, who gave much time and effort in supplying hæmatological material and information. In this regard special thanks are tended to Dr. A. W. Pound, Director of Pathology, Brisbane General Hospital, Brisbane, for his assistance. I am particularly indebted to Dr. P. E. Hughes, of this department, for his co-operation in much of the basic work of this study.

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ELECTROCARDIOGRAPHIC CHANGES IN TREATED HYPERTENSION[1]

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SUMMARY

The electrocardiograms of 143 patients treated with hypotensive drugs for two to six years have been analysed.

Improvement has occurred in 47% of cases; 35% of patients have remained unchanged, and 18% have deteriorated. The number of improved electrocardiograms was slightly higher in those treated for two to three years. Improvement was usually apparent within a year of treatment, but was not confined to this period.

In 17 cases the electrocardiogram was normal before treatment, and in 28 cases it was normal after treatment.

There was a small but significant reduction in the *QRS* voltage in leads reflecting left ventricular potentials.

In 74 cases depression of the *S-T* segment was seen before treatment. The degree of this was reduced in 54 cases after treatment.

Of 76 patients with inversion of the *T* waves in leads reflecting left ventricular potentials, 20 showed considerable improvement after treatment, the *T* waves becoming flattened or normal in all leads.

Follow-up of a 1953 series of 75 cases revealed that 24 patients have since died, of whom 14 had previously shown electrocardiographic improvement.

METHONIUM compounds have been used for the treatment of hypertension for six years, and many publications have referred to their indications and value (Smirk, 1954; McMichael, 1952; Morrison, 1953; Smirk *et alii*, 1954; Freis *et alii*, 1954). Smirk and Alstad (1951) reported that methonium treatment led to a reversion of the abnormal electrocardiogram of hypertension in the direction of normality, and Doyle (1953) described the electrocardiographic changes in 75 treated patients from the Medical Unit of the Dunedin Hospital. The object of this communication is to present a follow-up study of Doyle's series and to describe the present electrocardiographic status of 143 unselected patients treated with hypotensive drugs for two to six years.

METHODS

All available patients who had completed two or more years of hypotensive therapy and who were still on treatment in June, 1956, were included in this study. The only exclusions were those who had received digitalis at the time of their original or present electrocardiogram. Some patients living at a considerable distance from the clinic were not available for repetition of their electrocardiogram.

Classified according to the retinal grading of Keith, Wagener and Barker (1939), the series comprised: 17 cases of Grade IV hypertension, 33 of Grade III, 79 of Grade II, and 14 of Grade I.

The hypotensive drugs employed have varied at different times. During 1951 and 1952 hexamethonium and occasionally pentamethonium were used, in most cases by subcutaneous injection. Pentapyrrolidinium was introduced in 1953, being given at first parenterally and later orally, and this drug remains the most extensively used in this clinic. Since 1954, the rauwolfia alkaloids (reserpine, rescinnamine and canescine) have played an important part in treatment and are used principally in combination with pentapyrrolidinium. Other more recent drugs are chlorisondamine ("Ecolid") (Smirk and Hamilton, 1956), and mecamylamine ("Inversine" (Smirk and McQueen, 1957).

Standard twelve lead electrocardiograms were recorded in the recumbent position. The

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earlier recordings in 1950 and 1951, although qualitatively comparable, showed differences in the voltages of the deflections which made it desirable to analyse them separately. Records since 1952 were made on one of two direct-writing machines which gave identical voltage responses.

Each electrocardiogram was analysed in detail, and where necessary, reference was made to the criteria of left ventricular hypertrophy of Sokolow and Lyon (1949). Progress was assessed according to the following classification :

1. *Improved.*—Electrocardiograms were included in this category : (*a*) if the voltage of the *QRS* complex in leads reflecting left ventricular potentials showed reduction, (*b*) if *S-T* segment depression disappeared or was considerably reduced, (*c*) if the amplitude of the *T* waves in leads reflecting left ventricular potentials increased, or if previously negative *T* waves became positive or showed considerable reduction in negativity.

2. *Deteriorated.*—Significant changes in the opposite direction from those just described were judged to indicate electrocardiographic deterioration. In certain cases, abnormal voltage increases and *S-T* segment depression were reduced after treatment but definite increases in the *T* wave inversion occurred. Such cases were considered to have deteriorated.

3. *Unchanged.*—This category included all cases in which there was no significant change in the electrocardiogram after treatment.

Results

The Electrocardiogram before Treatment

Seventeen patients (12% of the total) had no electrocardiographic abnormality before treatment, while 69 (49%) showed changes of advanced left ventricular hypertrophy. The remainder showed less severe electrocardiographic changes.

Voltage Increases.—For reasons already stated, the figures in this section refer only to electrocardiograms taken since 1952.

Seventy-three out of 87 patients showed voltage increases indicative of left ventricular hypertrophy in one or more leads. In 17 of these there were no other signs of left ventricular hypertrophy.

In 22 cases, considerable left axis deviation (R_1+S_3 greater than 25 millimetres) was present, but seldom the sole evidence of left ventricular hypertrophy. The mean sum of R_1+S_3 was 19·17 millimetres for 87 cases. The electrical axis of the heart was commonly horizontal or semi-horizontal, and in 50% of these cases the height of the *R* wave in aVL exceeded 11 millimetres. Again in only six cases was this the sole evidence of left ventricular hypertrophy. In 11 patients the electrical axis of the heart was vertical, in which case *T* wave and *S-T* segment changes when present were found in Leads II, III and aVF.

In the precordial leads the mean heights of the *R* wave in V_5 and V_6 were 23·42 and 19·81 millimetres respectively. In 33 out of 85 cases the upper limit of normal (26 millimetres) was exceeded in one or both these leads. In 49 cases (56%) the sum of Rv_5 and Sv_1 exceeded 35 millimetres (mean 40·9 millimetres) ; and in 65 cases (80%) the sum exceeded 35 millimetres in Rv_5 and Sv_2 (mean 49·41 millimetres).

S-T Segment and T Wave Changes.—Depression of the *S-T* segment usually accompanied advanced *T*-wave inversion, and in only six cases was it the only abnormality.

TABLE I

Electrocardiographic Classification following Hypotensive Treatment for Two or More Years

Classification	Number of Cases	Percentage
Improved	67	47
Deteriorated	26	18
Unchanged	50	35
Total	143	100

Inversion of the *T* waves in one or more of leads reflecting left ventricular potentials occurred in 76 of the 143 cases and was accompanied by increased voltage in 82% of cases. Commonly the *T* wave was inverted in Lead I, aVL (if the heart was horizontal), and in V_5 and V_6. This was so in 69 cases. The *T* wave was never abnormal or inverted in Lead I alone, and only on two occasions were the changes confined to aVL, and in only one instance to the chest leads alone.

Biphasic or flattened *T* waves were regarded as indicative of less severe hypertrophy, and various combinations of abnormalities were present in 15 cases. Again, changes were never confined to one lead.

The Electrocardiogram after Treatment

Changes in the Electrocardiogram as a Whole.—The electrocardiographic status following treatment is summarized in Table I.

Thus almost half the patients have maintained some degree of improvement following treatment

TABLE II

Electrocardiographic Changes Classified according to the Duration of Hypotensive Treatment

Classification	Duration of Treatment							
	Five or More Years		Four Years		Three Years		Two Years	
	Number of Cases	Percentage	Number of Cases	Percentage	Number of Cases	Percentage	Number of Cases	Percentage
Improved	7	39	10	30	25	58	25	52
Deteriorated	4	22	7	20	4	9	11	23
Unchanged	7	39	17	50	14	33	12	25
Total	18	—	34	—	43	—	48	—

for two or more years; 35% have remained unchanged (including 16 of the 17 cases in which there was no abnormality initially), and only 18% have deteriorated. In 28 cases no electrocardiographic abnormality was found after treatment.

The distribution of improved cases according to the duration of treatment is illustrated in Table II. The incidence of improvement has been slightly higher in the two and three year follow-up, but the number of deteriorations has been fairly constant throughout the period of observation.

Electrocardiographic Changes after One Year of Treatment.—Fifty-three patients in this series had electrocardiograms performed twelve months from the onset of hypotensive treatment. Twenty-five showed improvement on that occasion, of whom four later deteriorated from their original electrocardiogram. Twenty patients showed no change at one year; nine of these subsequently improved, and six deteriorated. Eight patients had deteriorated at one year; one of these improved later, and two returned to their original status.

Voltage Changes.—A statistical analysis of voltage changes was made on 87 patients for whom electrocardiograms were available taken on similar machines. The results were analysed by Dr. A. M. O. Veale and are presented in Table III.

It will be observed from these figures that there was no significant change in the voltage of Rv_5 and Rv_6, although all the other values decreased significantly. When, however, only the cases which were initially abnormal in voltage in Rv_5 and Rv_6 are considered (*i.e.*, *R* wave 26 millimetres or greater), the reduction in voltage in both these leads after treatment was significant at the 1 in 100 and 1 in 20 levels respectively. The mean fall in voltage for Rv_5 in these cases was 5·88 millimetres and for Rv_6, 5·47 millimetres. On the other hand, it was observed that for those cases in which the voltage of Rv_5 and Rv_6 was normal before treatment, there was a small but significant increase in voltage. Thus for Rv_5 the mean voltage before treatment was 15·73 millimetres and after treatment 18·23 millimetres, an increase of 2.50 millimetres, which was significant at the 1 in 20 level ($0·05>P>0·02$). For Rv_6 the corresponding figures before treatment were 16·21 millimetres and after treatment 17·94 millimetres, and the increase was significant at the 1 in 10 level ($0·1>P>0·05$).

It was also observed that for all cases analysed the variance ratios of Rv_5 and Rv_6 were significantly decreased ($0·05>P>0·02$), by which is meant that the scatter of voltage in

TABLE III.

Statistical Analysis of Voltage Changes in the Electrocardiogram Before and After Hypotensive Treatment

Lead	Means in Millimetres			*N*	*T*	Significance
	Before	After	Difference			
R_1+S_3	19·17	17·90	−1·27	87	2·1913	$0·05>P>0·02$
RaVL	13·38	12·09	−1·29	43	2·1172	$0·05>P>0·02$
Rv_5	23·42	22·67	−0·75	83	0·4839	$0·7>P>0·6$
Rv_6	19·81	19·82	+0·01	85	0·0077	$P>0·9$
Rv_5+Sv_1	40·90	36·23	−4·67	87	4·0644	$P>0·001$
Rv_6+Sv_2	49·41	41·23	−8·17	81	5·991	$P>0·001$

these leads was much less after treatment. On further analysis, this was found to be true only for those cases in which the pre-treatment voltage was abnormal; those cases which were

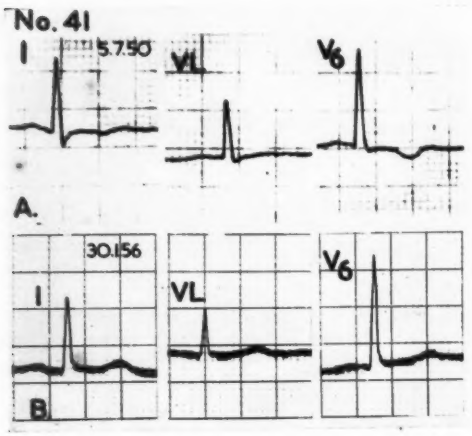

FIGURE I

normal before treatment had a greater dispersion after treatment.

Only six cases were classed as improved on the basis of voltage changes alone.

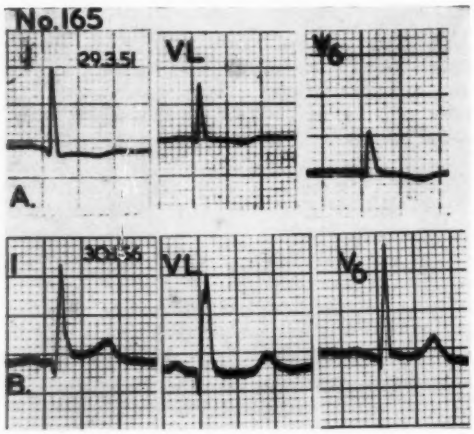

FIGURE II

T Wave and S-T Segment Changes.—*S-T* segment depression was commonly reduced after treatment: 54 out of 74 patients (73%) in whom this abnormality was originally present showed some degree of improvement. In 32 of the 55 cases in which extensive *T*-wave inversion remained after treatment, there was a reduction in the associated *S-T* depression.

Of the 76 cases in which inversion of the *T* waves was originally present in one or more

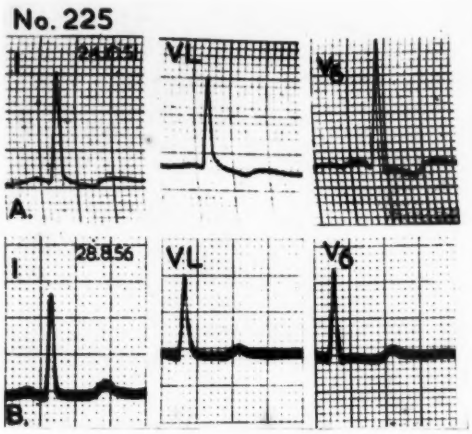

FIGURE III

leads reflecting left ventricular potentials, there were 20 examples in which all the inverted *T* waves became either positive or flattened. Where the *T*-wave inversion was considerable

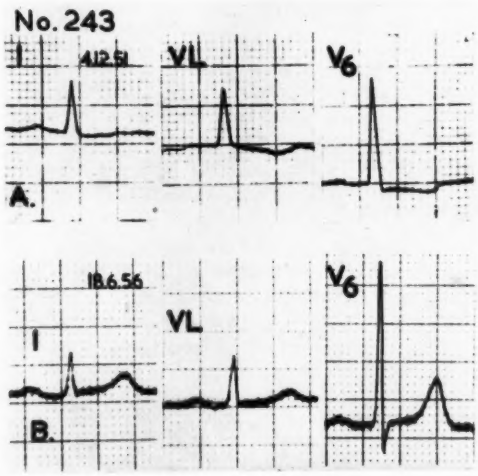

FIGURE IV

before treatment, it was, however, unusual for the electrocardiogram to return to complete normality. In 17 cases there was a definite increase in the negativity of the *T* waves.

In cases in which the electrical axis of the heart was horizontal, changes in the unipolar limb lead aVL were a sensitive index of alteration

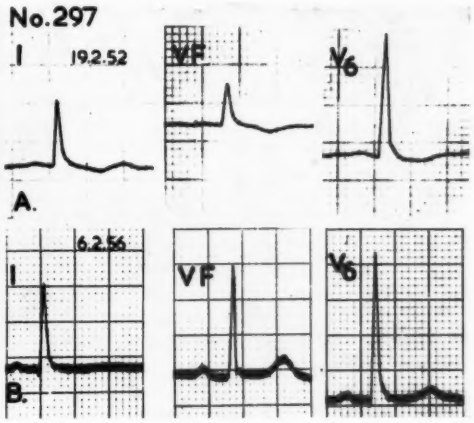

FIGURE V

in the pattern of left ventricular hypertrophy. In 10 out of 66 cases in which the *T* wave in aVL was originally inverted, the final record showed a

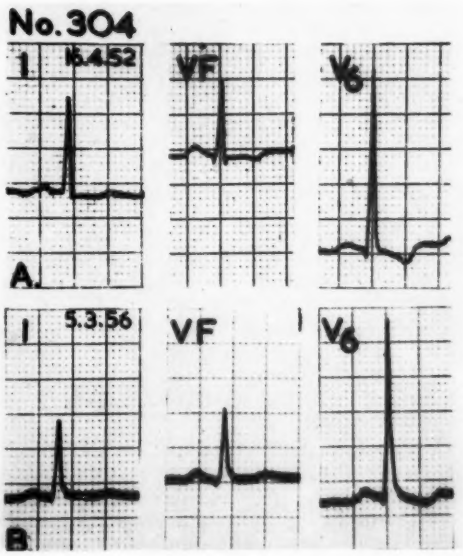

FIGURE VI

return to normality, and in eleven other instances there was improvement in the direction of normality.

An analysis of the heights of the *T* waves in Leads I, aVL and V6 in 85 cases showed that there was a small mean positive increase in

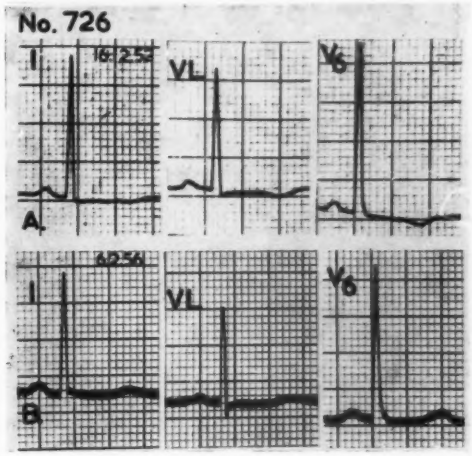

FIGURE VII

each of these leads. In the case of Lead I, the increase was significant at the 1 in 50 level ($0{\cdot}02 > P > 0{\cdot}01$).

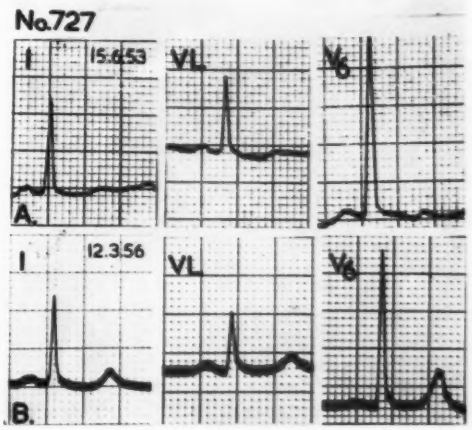

FIGURE VIII

Electrocardiograms illustrating some of the changes referred to are reproduced in Figures I to X. They relate to 10 patients; the case numbers correspond with those in earlier publications. The electrocardiogram marked "A" was taken before treatment, that marked "B" after treatment.

Follow-up of Doyle's 1953 Series

Doyle in *1953* reviewed the electrocardiographic changes in 75 patients treated with methonium compounds for periods of three to thirty months. Twenty-four of these patients have since died. Fourteen had shown electrocardiographic improvement, according to Doyle's analysis; ten were unchanged, and none had shown deterioration. Ten of these patients had further electrocardiograms before death: one improved further, two deteriorated (both had previously shown improvement), and seven were unchanged. The cause of death was cerebral hæmorrhage or thrombosis in twelve of these cases, uræmia in five, myocardial infarction in two, congestive heart failure in three (these patients lived at a distance, and control was inadequate), pneumonia and pulmonary œdema in one, and unknown in one.

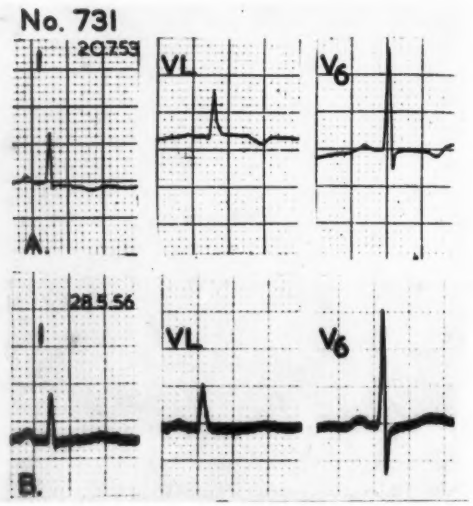

FIGURE IX

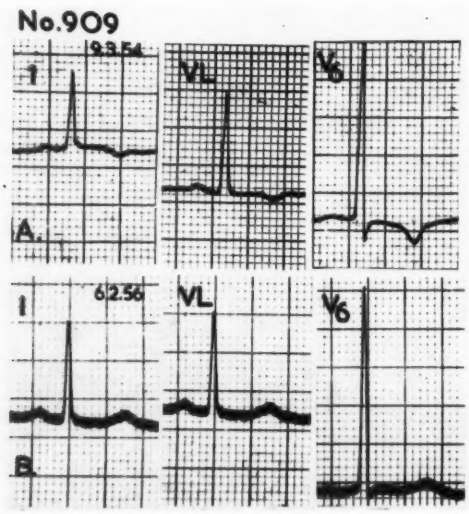

FIGURE X

Thirty-three of the remainder of Doyle's series have been analysed again. Eighteen of these were reported by Doyle to show electrocardiographic improvement in 1952. In 1956, eight retained improvement from the original electrocardiogram, six have returned to the original status, and four have deteriorated. The remaining 15 showed no change in Doyle's analysis. Seven continue to show no change in 1956; five have improved, and three have deteriorated.

DISCUSSION

Doyle (1953) stated that all abnormal electrocardiograms improved after hypotensive treatment for a year or more, and that improvements were directly related to the degree of blood pressure control achieved. As might be expected, the results of a longer investigation are less satisfactory, although the fact that 47% of patients have retained an improved electrocardiogram over a long period of observation and treatment is encouraging. The electrocardiogram in untreated hypertension usually tends to deteriorate in time (Canabal *et alii*, 1945), and it is therefore significant that only 18% of the treated patients in this series have regressed from their original record. The rate of change of the electrocardiogram was variable, but was usually obvious within a year of effective treatment. No clear correlation was possible between the drugs used and the electrocardiographic progress, for although there were a number of deteriorations following cessation of parenteral therapy, others first showed favourable changes after commencement of oral treatment.

Voltage reductions were small but statistically significant, the fall being greater according to the height of the *R* wave before treatment. Alterations in the *S-T* segments and *T* waves were more frequent and impressive changes, but in relatively few cases was the pattern of advanced left ventricular hypertrophy entirely lost, and in some it undoubtedly became worse. This regression did not always correlate with the clinical course, *e.g.* Case 400, a patient with malignant hypertension who lost his papill-

œdema, hæmorrhages and exudates, and returned to work with relatively few symptoms, but whose electrocardiogram has shown progressive left ventricular hypertrophy. Other patients have behaved similarly, with relief of symptoms and adequate blood pressure falls at clinic tests, but little change or even deterioration in the electrocardiogram.

Probably the most important factor influencing electrocardiographic and clinical improvement is the adequacy of blood pressure control. Other factors, such as the retinal grade and height of the basal blood pressure, while related to the severity of the original electrocardiographic changes, gave little indication of which patients would show electrocardiographic improvement or not. There was also no correlation between the age of the patient and improvement.

In other published results of hypotensive treatment varying electrocardiographic changes have been reported. Sieber *et alii* (1953), using mainly oral hexamethonium, found that 7 out of 26 patients showed reversion of *S-T* and *T* wave abnormalities to normal. Grob *et alii* (1953) using both orally and intravenously administered hexamethonium, found minor *T*-wave improvements in a moderate number of cases, but in no instance did an abnormal electrocardiogram become normal. In Morrison's series of patients treated with parenteral hexamethonium (Morrison, 1953), six patients, who had well marked left ventricular hypertrophy initially, showed " much improvement " after three to sixteen months. Harington and Rosenheim (1954) recorded improvement in one out of 16 cases, deterioration in four, and no change in 11. Goldsmith *et alii* (1955), also with severe cases, but using mainly oral treatment, found moderate electrocardiographic improvement in three, deterioration in one, and no change in three.

ACKNOWLEDGEMENTS

I wish to thank Professor F. H. Smirk for his valuable advice and criticism. Acknowledgement is also made to Dr. A. M. O. Veale for the statistical analysis, to Miss N. Richardson for secretarial help, to Mr. T. Wallis for the illustrations, and to Miss N. Cull and Miss V. Walsh for technical assistance.

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PHONOCARDIOGRAPHY IN THE ASSESSMENT OF MITRAL VALVE DISEASE[1]

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SUMMARY

Phonocardiograms of 250 consecutive subjects of mitral valve disease have been examined. The findings in mild mitral stenosis and in predominant mitral incompetence are described.

Seventy cases in which the state of the mitral valve was ascertained at operation have been analysed in more detail. The typical findings of " tight " mitral stenosis are described. Variations from this picture may occur where there is heavy mitral valve calcification, where severe pulmonary hypertension accompanies the development of a high pulmonary vascular resistance, and where there is significant aortic valve disease.

The importance of the group of cases with severe pulmonary hypertension is emphasized. Cases in this group are often misdiagnosed, either because the signs are wrongly interpreted as being due to mitral incompetence or because the presence of mitral stenosis as the underlying cause of pulmonary hypertension is not recognized.

The part played by phonocardiography in the diagnosis of these cases of mitral valve disease, and in the correct assessment of the state of the mitral valve in these and other cases, is stressed.

THE operation of mitral valvotomy for the relief of mitral stenosis is now well established, and its general indications are well known (Dexter *et alii*, 1954).

Nevertheless, it is the experience of every cardiac clinic that a high proportion of cases of mitral valve disease presenting for assessment are unsuitable for surgery. In many, mitral stenosis is present, but the valve narrowing has not reached that critical level at which serious symptoms are liable to follow (Gorlin and Gorlin, 1951). Symptoms are due to other factors, which will not be relieved by mitral valvotomy. In others, all too many in number, symptoms may be severe, and yet surgery can offer no real help because the mitral valve is significantly incompetent. Consideration of the history, search for signs of pulmonary congestion and heart failure and information obtained from cardiac catheterization may give a clear indication of the severity of physiological upset, but will not give an absolute indication of the anatomical state of the mitral valve. It is the latter that determines the surgeon's ability to perform a useful operation. Even direct pressure measurement from the left atrium may not yield unequivocal information (Burchell, 1956). It is important therefore to consider what other features can help in the proper selection of patients.

Clinical auscultation remains an important method of assessment which, while simple, may be very rewarding, if carried out with careful attention to detail. The clinical phonocardiogram provides an extension of auscultation. It records the heart sounds and murmurs, much as heard through the stethoscope, but provides a permanent record in which the intensity, shape and timing of heart sounds and murmurs can be accurately measured and analysed.

Phonocardiography has been a routine method of assessment of cases of mitral valve disease at the Alfred Hospital for the past three years. The present paper is an examination of the information it has afforded in 250 consecutive cases, especially in 70 cases in which the state of the mitral valve was determined at operation, and in a further seven cases in which examination was made at autopsy.

MATERIAL AND METHODS

The records were examined of a consecutive series of 250 cases of mitral valve disease in which phonocardiography was performed. The clinical groups are given in Table I. In seven cases the state of the mitral valve was determined subsequently at autopsy. In 78 cases the patients were subjected to operation, but of these the findings were incomplete in eight cases.

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A detailed analysis was made of the phonocardiograms in the remaining 70 cases, in which satisfactory examination of the valve had been made at operation. The following features were noted: first heart sound at the apex, apical systolic murmur, opening snap, third heart sound, mid-diastolic murmur and presystolic murmur; systolic murmur over the right ventricle; second heart sound in the pulmonary area; and associated murmurs. Satisfactory measurements of the time intervals *Q*–1 and 2–OS corrected to a heart rate of 75 per minute were obtained in 23 cases (Wells, 1954).

TABLE I

Mitral Valve Disease: Clinical Groups

Group	Number of Cases
Mild mitral stenosis	35
Predominant mitral incompetence	80
Predominant aortic valve disease ..	21
Young cases	19
Miscellaneous	10
Operated cases..	78
Autopsy cases	7
Total	250

The phonocardiograms in the first 100 cases were recorded on a Sanborn Stethocardiette, and in the remainder on an N.E.P. multichannel recorder, using two phonocardiographic channels. Reference tracings used were electrocardiogram and carotid pulse, with jugular venous tracing where indicated.

RESULTS

Non-Operated Series

Of the cases in the non-operated series the findings in the two main groups are as follows:

Mild Mitral Stenosis.—In this group the outstanding feature is the pre-systolic murmur, often loud, in contrast to a relatively soft mid-diastolic murmur. There is usually a loud first sound. The opening snap may or may not be recorded; occasionally it is strikingly loud at the base.

Mitral Incompetence.—In "pure" mitral incompetence the outstanding feature is a loud apical pan-systolic murmur, often accompanied by a thrill. The first sound is not loud or delayed. The aortic element of the second sound is often obscured by the murmur, but the pulmonary element may be loud in the pulmonary area and moderately widely split. There is usually a third heart sound at the apex (Figure I) (Brigden and Leatham, 1953).

Cases of pure mitral incompetence are uncommon, but the combination of stenosis and incompetence, the latter predominant, is the most frequent finding in this series. These cases show signs which are a modification of the signs of pure incompetence; as a number are included in the operated series they will be discussed later.

Operated Series

In the operated series the anatomical state of the mitral valve, and the presence or absence of mitral incompetence were determined by the same thoracic surgical team. It should be realized that some of the cases, especially those with predominant incompetence, represent cases in which clinical signs have been somewhat exceptional and sufficient to lead to exploratory cardiotomy.

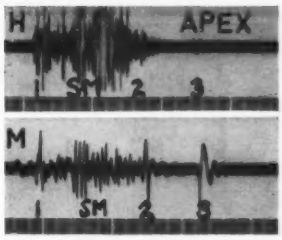

FIGURE I

Pure mitral incompetence. Stethoscopic (M) and logarithmic (H) records at the apex, not simultaneous, showing the pan-systolic murmur (SM) and the clear third heart sound (3). Time markers here and in subsequent tracings every 0·04 second

The 70 cases have been divided into three groups according to the surgeons' findings (Table II). The presence or absence of significant calcification is noted. The phonocardiographic findings are summarized in Table III.

1. *Tight or Predominant Mitral Stenosis.*—In this group were 51 cases, 15 with significant calcification. Thirty-eight patients were in sinus rhythm, and 13 had auricular fibrillation.

The typical features found in this group are as follows:

At the apex the first heart sound contains a loud element of fairly high frequency occurring at the end of the first sound, 0·08 second or later than the *Q* wave of the electrocardiogram. The louder elements of the normal first sound

occur not later than 0·06 second after the *Q* wave (Wells, 1952); hence the first sound in mitral stenosis is said to be delayed. There is usually no systolic murmur, but sometimes a few rather low-pitched early systolic vibrations follow the first sound. The second sound at the apex is unremarkable; following it, from 0·06 to 0·12 second later, there is usually, but not invariably, an opening snap. Shortly after this the mid-diastolic murmur commences, in some cases with a well defined onset but never with a clear third heart sound. The mid-diastolic murmur is long, and in those cases in sinus rhythm it has moderate pre-systolic accentuation before the next first heart sound. The opening snap is well recorded within the apex at the sternal edge and may be clearly seen at the base, especially the aortic area. The second heart sound in the pulmonary area is only exceptionally very loud, nor is it usually split on held expiration. It commonly, however, splits into aortic and pulmonary components with inspiration, though rarely wider than 0·04 second. In about half the cases the pulmonary element is as loud as or louder than the aortic. These points are illustrated in Figure II.

TABLE II

Operation Series

Findings	Number of Cases
Mitral stenosis "tight" or predominant:	
Without calcification	36
With calcification	15 } 51
Mitral stenosis not "tight":	
Without calcification	5
With calcification	2 } 7
Mitral stenosis with significant incompetence:	
Without calcification	8
With calcification	4 } 12
Total	70

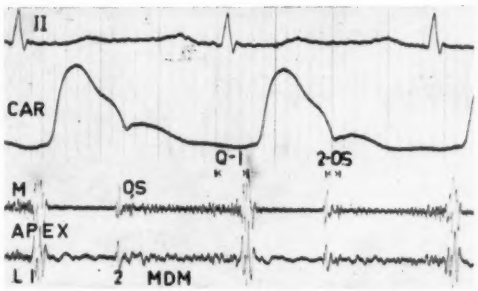

FIGURE II

"Tight" mitral stenosis. Simultaneous low frequency (L) and medium frequency (M) records taken at the apex, showing the loud delayed element of the first heart sound (1), second sound (2), opening snap (OS), and mid-diastolic murmur (MDM) with pre-systolic accentuation. There is no clear third heart sound. Reference tracings are the electrocardiogram (II) and carotid pulse (CAR). *Q*-1 interval = 0·11 second, and 2-OS interval = 0·05 second

Certain variations from this typical picture were encountered. The first sound had a loud and delayed element except in three with heavy calcification. Ten patients had a slight early

TABLE III

Phonocardiographic Findings in Operation Series[1]

Group	Total	Calcification	Apical 1st H.S.			Apical S.M.			Opening Snap			3rd H.S.			Apical M.D.M.			Apical P.S.M.			S.M. L.S.E.		2nd H.S. in Pul.				Other Murmurs	*Q*1–2OS		
																							Split		Loud					
			Loud	Normal	Soft	Absent	Early	Pan	+	Soft	Absent	Soft	Clear	Long	Short	Soft	Absent	Total N.S.R.	Present Moderate	Marked	Soft	Pan	Insp.	Const.	+	++		Measured	+1 or More	0 or Less
M.S. "tight" or predominant	51	None 36	35	1	—	24	9	3	33	3	—	10	2	35	—	—	1	30	22	1	4	4	18	2	14	1	3 A.I.	14	12	2
		+ 15	7	5	3	10	1	4	9	4	2	4	2	12	—	1	2	8	5	—	—	4	9	2	10	3		4	4	—
M.S. not "tight"	7	None 5	5	—	—	4	1	—	4	—	1	—	1	—	—	5	—	3	—	3	—	—	1	—	1	—		1	—	1
		+ 2	2	—	—	2	—	—	1	—	1	1	—	1	1	—	1	2	—	2	2	—	2	—	—	—		—	—	—
M.S. with significant incompetence	12	None 8	8	—	—	3	4	1	4	3	1	1	5	3	5	—	—	5	—	2	2	—	5	—	5	—	1 A.S.+I. 1 A.I.	3	1	2
		+ 4	1	—	3	—	1	3	—	1	3	—	2	1	3	—	—	2	—	—	2	—	1	—	1	1		1	—	1

[1] "M.S."=mitral stenosis; "H.S."=heart sound; "S.M."=systolic murmur; "M.D.M."=mid-diastolic murmur; "P.S.M."=pre-systolic murmur; "L.S.E."=left sternal edge; "Pul."=pulmonary area; "*Q*1–2OS", see text; "N.S.R."=normal sinus rhythm.

systolic murmur. In a further seven cases a pan-systolic murmur was recorded at the apex, but in all it was as loud or louder in the fourth left interspace at the sternal edge. These, with one other, constitute a special group which will be considered separately. An opening snap was recorded in all non-calcified cases; in the cases with calcification it was present in nine, but barely discernible in four and absent in two.

In 14 cases the mid-diastolic murmur started a little loudly, but no clear third sound was seen. In four cases a clear third heart sound was recorded medially over the right ventricle; in two it was also recorded at the apex. All these cases fell into the special group with a loud pan-systolic murmur at the left sternal edge. The mid-diastolic murmur was long in all uncomplicated cases, but of the eight patients of this special group, three had no mid-diastolic murmur, only a few pre-systolic vibrations being visible, and in one case it was soft and variable. Of the 38 patients in sinus rhythm, three-quarters had some pre-systolic accentuation of the diastolic murmur, but this was striking in only one case, in which there was a moderate degree of aortic incompetence.

In four cases there was a slight systolic murmur recorded in the fourth left interspace, early in timing and not loud. Eight patients, however, had a loud long pan-systolic murmur; in seven of these it was well recorded at the apex. Seven of these patients were in sinus rhythm. The group is discussed later.

The second heart sound in the pulmonary area was slightly accentuated in 24 cases, but was unduly loud in only four, which all fell into the special group already mentioned. Contrary to older teaching, the second sound was split on expiration (to 0·03 second or more) in only four cases, although it would usually split on inspiration.

2. *Mild Mitral Stenosis.*—In this group of seven cases, significant valve calcification was present in two. Five patients were in sinus rhythm, and two had auricular fibrillation. The signs were not fundamentally different from those of "tight" stenosis, but the mid-diastolic murmur was soft in six cases, and of the five patients in sinus rhythm, all had a pre-systolic murmur much louder than the mid-diastolic. An opening snap was present in five cases. In one case it was exceptionally loud at the base, particularly the aortic area.

3. *Mitral Stenosis and Incompetence.*—This group comprised 12 cases, four with significant valve calcification. Seven patients were in sinus rhythm, and five had auricular fibrillation. Patients with this lesion tend to show a combination of the signs of pure mitral incompetence with those of stenosis. Typically the first sound is neither loud nor delayed. There is a systolic murmur, which is not necessarily loud but is usually high in frequency and is pan-systolic, running right up to the second sound. There is usually only a tiny opening snap, and this is followed by a mid-diastolic murmur which starts loudly, commonly with a discrete "third heart sound". The murmur tends to be short, fading away quickly; in cases with sinus rhythm there may be little or no pre-systolic murmur.

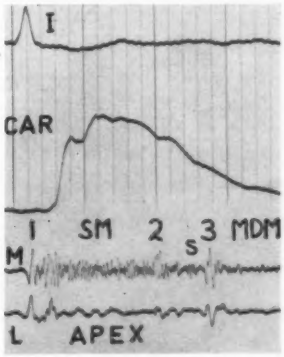

FIGURE III

Mitral stenosis and incompetence. Simultaneous low frequency (L) and medium frequency (M) records taken at the apex, showing the normal first sound (1), the pan-systolic murmur (SM) running up to the second sound (2), an opening snap (S), and a third heart sound (3), which is followed by a short mid-diastolic murmur (MDM)

In this group of twelve cases in which exploration of the mitral valve was undertaken, only one patient showed all these features (Figure III). In others exploration had been thought worthwhile because auscultatory signs were not typical of incompetence. The first sound, for instance, was loud in all cases except three of the four with calcification, and in one instance was delayed up to 0·10 second at a heart rate of 75 per minute. Four patients had a pan-systolic murmur maximal at the apex. In five others, however, there was only a moderate early systolic murmur, and in three cases there was no apical systolic murmur. A clear opening snap was recorded in four non-calcified cases. In four others only a tiny

snap could be made out at the apex, but in two of these it was loudly recorded at the base. Seven patients had an apical third heart sound; in four others this was absent. In eight cases the mid-diastolic murmur was loud although short, in four it was fairly long. Of the seven patients in sinus rhythm, two had a definite pre-systolic murmur: this is in contrast to the findings of Wood (1954). In no case was a systolic murmur recorded at the left sternal edge louder than at the apex. In six cases the second heart sound was accentuated at the pulmonary area and split on inspiration. In one case it was very loud but not clearly split.

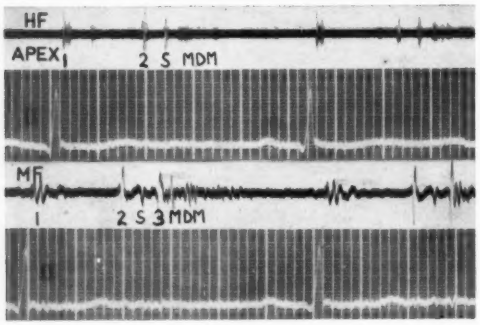

FIGURE IV

Mitral stenosis and incompetence. Stethoscopic (MF) and logarithmic (HF) records taken at the apex, not simultaneous, showing no clear systolic murmur and an opening snap (S), but a clear third heart sound (3) and a relatively short mid-diastolic murmur (MDM), which has no pre-systolic accentuation even in sinus rhythm

Although as a group all the signs of mitral stenosis could be found in one case or another, yet in no one case did a combination of all findings suggest "tight" mitral stenosis. In every case the patient had some features suggesting the presence of mitral incompetence. For instance, one patient with a loud first sound, no systolic murmur and a fairly clear opening snap had, in contrast, a loud third heart sound and a short mid-diastolic murmur which faded away rapidly without pre-systolic accentuation (Figure IV).

Autopsy Series

In the autopsy series of seven cases, the state of the mitral valve was examined at autopsy. One case was that of a young man who died of acute rheumatic carditis without established valve lesions. One woman of fifty-four years had the signs of mitral stenosis and incompetence as previously outlined, and autopsy findings agreed with this.

Five subjects had "tight" mitral stenosis. In two, a loud murmur over the right ventricle had been recorded in association with tricuspid incompetence. The physical signs at the apex were similar to those of the group with this murmur in the operated series.

The remaining three subjects had established aortic valve disease of significant degrees, stenosis in two, stenosis and incompetence in the other. Phonocardiographic signs at the apex resembled those of mild mitral stenosis with a soft mid-diastolic murmur and well marked pre-systolic accentuation. In these cases, as in the cases with high pulmonary resistance, the signs of "tight" mitral stenosis had been modified.

DISCUSSION

In the assessment of subjects of mitral valve disease with regard to their suitability for operation, there is no single piece of information which will provide an unequivocal answer to the problem. All available evidence must be considered in reaching a decision. Nevertheless, clinical auscultation may provide extremely valuable clues which the phonocardiogram records with precision.

In the great majority of subjects with "tight" mitral stenosis, the auscultatory signs are typical, and have been described by various authors, including Wood (1954). The phonocardiographic features have been dealt with by Leatham (1952) and Ongley *et alii* (1955). Their findings are confirmed in this investigation.

A study of the time relationships of the first heart sound and opening snap has been made by Messer *et alii* (1951), who showed that the shorter the previous cycle length, the greater the delay of the first heart sound and the shorter the gap between the second sound and the opening snap. They related this phenomenon to the diastolic pressure gradient across the mitral valve. This observation has been used by Wells (1954) as a phonocardiographic test for the severity of mitral stenosis. Wells measures the time from the onset of the *Q* wave of the electrocardiogram to the loud element of the first heart sound (*Q*–1 interval) and that from the aortic element of the second heart sound to the opening snap (2–OS interval). The measurements are made at a heart rate of 75 per minute (previous cycle length 0·8 second), or corrected to that rate, and are expressed in hundredths of a second. An index (*Q*1–2OS) is then derived by subtracting "corrected 2–OS" from "corrected *Q*–1". The more positive the index, the tighter the stenosis. Wells found values of −1 to +5 or more in cases of tight stenosis.

In this series there were 18 cases of tight mitral stenosis in which this test could be satisfactorily applied. Values ranging from 0 to +6 were found, two cases having the borderline value of 0. Of the group of mild mitral stenosis, one case had a value of 0. Of four cases of mitral incompetence, two had negative values of −2 and −4 respectively, but one was borderline at 0, and in the other the value of +2 suggested stenosis. From this admittedly small number of cases, it would appear that although this test may give some general indication it is not absolutely reliable. The effect of successful valvotomy in a case in which the pre-operative value is +6 is shown in Figure V. After operation the value is 0.

It should be pointed out, however, that the signs of "tight" mitral stenosis become modified under certain circumstances. The first of these is the presence of significant mitral valve calcification (Wynn, 1953). Thus in three such cases the first sound was not loud, and in these and three others with calcification the opening snap was absent or very small. Due allowance for these points must be made when assessing cases which have obvious valve calcification on screening. (In one case of tight mitral stenosis subsequent to this series, the opening snap was absent although screening showed no mitral valve calcification. At operation the valve was exceptionally fibrous and rigid.) It is now generally recognized that the presence of an opening snap depends upon the mobility of the mitral valve cusps and the movement of the valve diaphragm. It is probable that the loud and delayed element of the first heart sound depends also to some extent upon these factors.

The second set of circumstances under which physical signs may be modified is illustrated by that interesting and important group of ten cases of "tight" stenosis, eight in the operated series and two examined *post mortem*, in which a loud pan-systolic murmur was recorded at the left sternal edge in the third and fourth intercostal spaces. In nine of the ten cases it was well recorded, though not so loudly, at the apex. In these cases the patients all had evidence of considerable pulmonary hypertension with increased pulmonary vascular resistance. The importance of the group lies in the fact that the physical signs may be wrongly interpreted as those of mitral incompetence or, indeed, that the presence of mitral valve disease may be entirely unsuspected.

This may occur because in these cases the apical mid-diastolic murmur of mitral stenosis may be absent or very difficult to detect. In sinus rhythm it may be represented only by a few presystolic vibrations. It thus tends to resemble more closely the murmur found in mild mitral stenosis than that of combined stenosis and incompetence. The diagnosis of mitral incompetence, however, may be entertained because of the loud systolic murmur extending out to the apex.

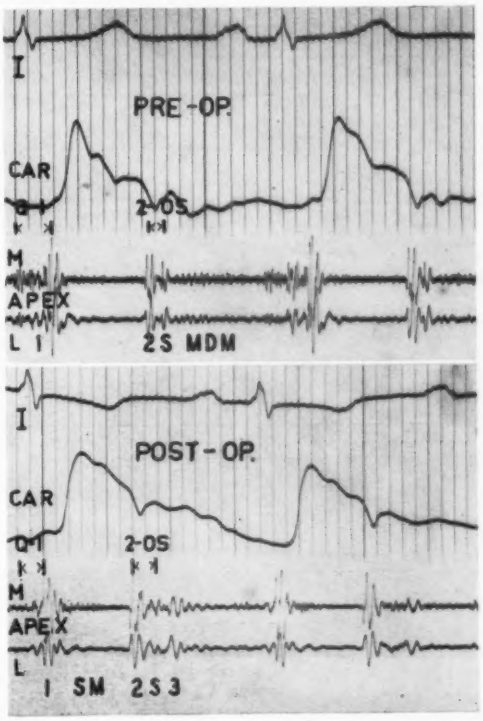

FIGURE V

Pre-operative and post-operative records in "tight" mitral stenosis. Low frequency (L) and medium frequency (M) taken at the apex before and after operation. S=opening snap. Pre-operatively the *Q*-1 interval is 0·11 second and the 2-OS interval is 0·05 second. *Q*-1—2-OS=+6. Post-operatively the *Q*-1 interval is 0·07 second and the 2-OS is 0·07 second. *Q*-1—2-OS=0. There is now an apical systolic murmur (SM), and a third heart sound (3)

The phonocardiographic features of such a case are illustrated in Figure VI. The jugular venous tracing shows tall *a* and *v* waves, with some filling-in of the systolic descent in late systole, especially on inspiration. There is a loud pan-systolic murmur at the left sternal edge, well recorded also at the apex. Here the first sound is not loud, there is only a tiny sound in the position of the opening snap, and the

diastolic murmur of mitral stenosis is represented by a few vibrations in presystole. This man of 37 years had a history of rapidly increasing disability and recent congestive failure. Because of urinary findings suggesting a nephritic lesion, and of an electrocardiogram interpreted as that of right bundle branch block rather than right

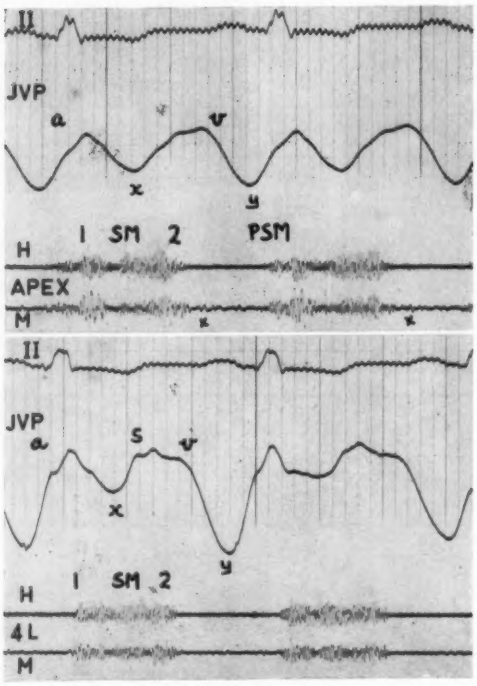

FIGURE VI

Mitral stenosis with severe pulmonary hypertension. The upper tracing shows medium frequency (M) and high frequency (H) records taken at the apex, showing a pan-systolic murmur (SM) and a pre-systolic murmur (PSM). The first heart sound is not loud, the opening snap is probably represented by soft sound marked X. The jugular phlebogram (JVP) shows *a* and *v* waves, with a shallow systolic descent (x) and deeper diastolic descent (y). The lower tracing shows records taken at the same amplification in the fourth left interspace at the sternal edge (4L) during inspiration. The loud pan-systolic murmur (SM) does not change, but the jugular phlebogram shows a filling-in of the systolic descent by a positive wave (S), indicating tricuspid incompetence.

ventricular hypertrophy, with a plain chest X-ray film which did not show discernible left auricular enlargement, the diagnosis had been made of cardiopathy possibly secondary to *polyarteritis nodosa*. The phonocardiographic features pointed to the presence of mitral valve disease, and at operation tight mitral stenosis was found.

The genesis of the pan-systolic murmur recorded over the right ventricle may not always be obvious. Although in the three cases with auricular fibrillation a single systolic wave in the jugular venous pulse made it clear that there was tricuspid incompetence, the seven cases in sinus rhythm all showed tall *a* and *v* waves, without a single systolic wave. The phlebogram, however, showed a shallow mid-systolic descent and a deeper early-diastolic descent. On inspiration the mid-systolic descent tended to disappear, a positive systolic wave appearing in the later part of systole (see Figure VI). These findings probably indicate lesser degrees of tricuspid incompetence, as it presents in normal rhythm. Similar findings have been reported by Müller and Shillingford

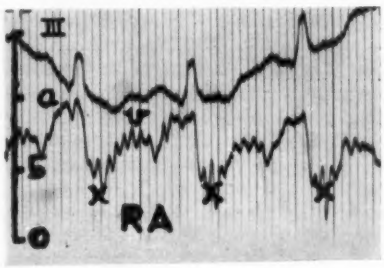

FIGURE VII

Right auricular tracing in a case of mitral stenosis with severe pulmonary hypertension (same case as in Figure VI), showing sharp systolic descent (x), although the systolic murmur at the left sternal edge illustrated in Figure VI persisted. Right auricular pressure scale graduated every 5·0 millimetres of mercury above the sternal angle

(1954). It is becoming increasingly clear that the murmur of tricuspid incompetence may be present when other signs of this lesion—namely, a systolic wave seen in the venous pulse and felt at the liver edge—may be minimal. It has been surprising to note in these cases, for instance, that when the venous pressure level has been reduced by treatment and a well-marked early-systolic descent has been recorded in the phlebogram, and even in the right auricular pressure tracing (Figure VII), the systolic murmur over the right ventricle has yet remained quite intense. This murmur is similar to that recorded for some two years before death in a case of autopsy-proven primary pulmonary hypertension, in which the jugular phlebogram showed identical features (Figure VIII).

The main practical point is that in many cases, especially in sinus rhythm, in which this systolic murmur indicates right ventricular dilatation under the stress of severe pulmonary hypertension, the jugular venous pulse may not show the single large systolic wave usually regarded as the hallmark of tricuspid incompetence. The site of origin of the murmur may then be misinterpreted and its true significance missed.

In this connexion it may be pointed out that in cases presenting as pulmonary hypertension of unknown ætiology, in which no mid-diastolic murmur is audible at the apex, the phonocardiogram may provide a clue to the presence of

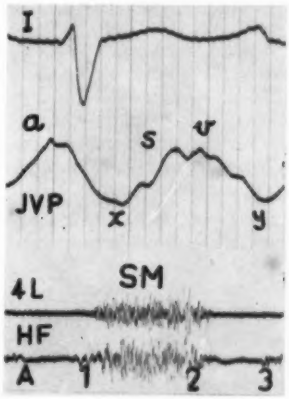

FIGURE VIII

Primary pulmonary hypertension. Simultaneous high frequency (HF) records taken at the fourth left interspace (4L) and apex (A), showing the loud pan-systolic murmur (SM) and third heart sound (3). The jugular phlebogram (JVP) shows tall *a* and *v* waves with only a small positive mid-systolic wave (S)

mitral valve disease by recording an opening snap. Electrocardiographic and X-ray evidence of left auricular hypertrophy and of mitral valve calcification may complete the diagnosis.

In all but one of these cases there was clinical and electrocardiographic evidence of right ventricular hypertrophy. The finding of a strong pattern of right ventricular hypertrophy in a case of suspected mitral incompetence should lead to a careful reexamination of the clinical picture. Nevertheless, the finding does not invariably point to the presence of tight stenosis, as it has occurred in our series in two proven cases of mitral incompetence with associated pulmonary hypertension.

Cardiac catheterization will demonstrate the presence of considerable pulmonary hypertension, as was shown in the five cases in which the subjects were catheterized. The finding of a high pulmonary capillary pressure does not in itself demonstrate the state of the mitral valve, nor does the form of the pulmonary capillary tracing. Left-sided heart catheterization, with measurement of the pressure gradient across the valve, will provide further information, but the technique is difficult and potentially dangerous. In the presence of the phonocardiographic signs already outlined exploratory thoracotomy is justified and is more likely to provide a satisfactory solution.

The existence of this group is recognized, among others, by Wood (1954) and mentioned by Mounsey and Brigden (1954). Nevertheless, it is thought to be of sufficient importance to draw attention once again to its features.

One somewhat confusing point may be the recording of a clear third heart sound, as was found in four of the present cases. In two cases it was loudest over the right ventricle, but in the other two cases it was well recorded at the apex. It was, however, never accompanied by the loud but short mid-diastolic murmur found in mitral incompetence. Indeed, where the signs of tight mitral stenosis are modified by the presence of pulmonary hypertension, the diastolic murmur resembles that of mild mitral stenosis rather than that of mitral incompetence.

A third set of circumstances in which the signs of tight mitral stenosis may be atypical is the presence of significant aortic valve disease. Of the operated subjects of tight stenosis three had the murmur of aortic incompetence, but in only one was this hæmodynamically significant. In this case auscultatory signs at the apex resembled those of mild stenosis, as there was a relatively soft mid-diastolic murmur and a loud presystolic murmur. As already pointed out, similar signs were present in three autopsy cases of tight stenosis with significant aortic valve disease. The associated aortic lesion tends to modify the auscultatory signs of mitral stenosis, so that signs of severity are minimized. As in cases of high pulmonary resistance, this may be because cardiac output and therefore mitral valve flow is low, or because left ventricular diastolic pressures are higher. It should be borne in mind in the assessment of any case with significant aortic valve disease.

The subjects with predominant mitral incompetence showed some variations from the typical features described. It is evident, for

instance, that the first sound may be loud and delayed in mitral incompetence, that there may be a clear opening snap and that there may be no audible or recordable apical systolic murmur. A third heart sound and short mid-diastolic murmur are usually found but are not invariable. However, in cases in which there is a strictly apical pan-systolic murmur with a third heart sound and a short mid-diastolic murmur, mitral incompetence is almost certainly present, even in the presence of pulmonary hypertension.

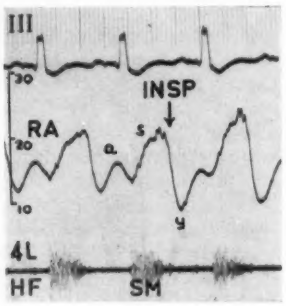

FIGURE IX

Tricuspid incompetence. Tracing taken at cardiac catheterization, showing a high frequency (HF) recording at the fourth left interspace (4L) in a case of mitral stenosis with severe pulmonary hypertension with tricuspid incompetence. In this case the loud pan-systolic murmur (SM) decreases on inspiration (INSP), although the positive systolic wave (S) in the right auricular tracing (RA) increases in height. Mitral incompetence was absent at operation. Right auricular pressure in millimetres of mercury above sternal angle

One of the most important points arising from this study is the necessity for differentiation between the pan-systolic murmur of mitral incompetence and that heard over the right ventricle in tricuspid incompetence. The surest method is careful note of the site of maximum intensity. In recent years the effect of respiration on these murmurs has been studied in some detail. Müller and Shillingford (1954) have demonstrated that the murmur of tricuspid incompetence may increase on inspiration. Although this is often so, in some cases the effect is not clear cut. The murmur may be so intense as to be virtually unvarying. In cases with auricular fibrillation, the effect of varying cycle lengths overshadows the respiratory effects. In still other cases there is actual decrease in inspiration. Thus in one subject in normal rhythm, subsequent to this series, with high pulmonary resistance, frank tricuspid incompetence and an intense systolic murmur over the right ventricle but extending out to the apex, careful records showed that the murmur decreased on inspiration (Figure IX). At operation very tight mitral stenosis was found, with no trace of mitral incompetence. One subject in the operated series who showed this change has recently died of unrelated malignant disease. No organic disease of the tricuspid valve or other structural lesion other than a dilated right ventricle was found to account for the systolic murmur.

If cases of tight mitral stenosis with high pulmonary resistance and a loud systolic murmur over the right ventricle are not to be misdiagnosed, it must be recognized that there need not necessarily be a large systolic wave in the neck veins in tricuspid incompetence, at any rate in the presence of normal rhythm. Nor must this murmur necessarily increase on inspiration, if its origin is to be traced to the right ventricle, and its significance recognized.

Splitting of the second heart sound has in the past been regarded as an important additional sign of mitral stenosis. In this series the second sound was only occasionally significantly split on expiration, although it could be shown to split on inspiration in over half the cases. The opening snap was often clearly recorded at the pulmonary and aortic areas and has obviously led to confusion with the second heart sound in the past. Where there is true wide splitting of the second heart sound (0·06 second or more), atrial septal defect must be suspected. In cases in which there is clinical difficulty in differentiating these sounds, the phonocardiogram, as shown by Margolies and Wolferth (1932), provides a ready and unequivocal solution.

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LEFT VENTRICULAR HYPERTROPHY : THE DETERMINATION OF L.V. WEIGHT[1]

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SUMMARY

From an autopsy study of the weights of the isolated ventricles in 102 adults hearts, an analysis was made of the accuracy with which total ventricular weight, total heart weight and left ventricular thickness permitted the prediction of the left ventricular mass and the distinction between its normality and hypertrophy.

Normal figures were derived from the literature and from 16 normal cases in this series. The effsect of formalin fixation were studied in 11 cases.

From the results it was concluded that, in the absence of right ventricular hypertrophy, total ventricular weight was a reliable guide to left ventricular weight. Total heart weight was less reliable and involved an error in prediction of between $\pm 10\%$ and $\pm 20\%$, which renders it but a crude indicator of left ventricular hypertrophy. Left ventricular thickness, whether measured at one or many sites, showed no useful correlation with the ventricular mass.

ALTHOUGH considerable attention has been devoted to the study of left ventricular hypertrophy, to its ætiology, its clinical diagnosis and its prognostic implications, it is rarely that investigators have employed the direct measurement of isolated left ventricular weight as a foundation for their work. The lack of critical analysis of the accuracy of the more indirect method by which hypertrophy has been predicted is no doubt reflected in the diversity of opinions expressed on the efficacy of the various aids to diagnosis and particularly on the value of electrocardiography in this field. It was a preliminary to such an electrocardiographic study that the present work was undertaken.

The determination of hypertrophy involves, firstly, the accurate measurement of the mass of the relevant organ and, secondly, the definition of an upper limit of normality.

The direct measurement of left ventricular weight can of course be made only at autopsy and demands the somewhat tedious removal of all non-ventricular tissues. Even so, earlier workers who did this (Muller, 1883; Lewis, 1914; Cotton, 1917; Herman and Wilson, 1922) reached no agreement as to how the interventricular septum should be apportioned to the respective ventricles. Various methods of division and summary allocation were employed. Fulton and his associates (1952) demonstrated that the difficulty could best be solved by including the septum with the left ventricle after the amputation of the free wall of the right ventricle—a relatively accurate dissection. Jones (1953) endorsed the view that septal division was open to misleading inconsistency. The indirect autopsy methods most commonly used for assessing left ventricular weight have been total heart weight and/or left ventricular thickness.

While total heart weight has the great advantage of simplicity, it includes a variable weight of extraventricular tissue. No adequate statistics are available to show the accuracy with which left ventricular weight can be so predicted.

Left ventricular thickness must be considered an unreliable indicator of the ventricular mass. In the first place, as Shohet and others (Shohet, Taub and Kupersmith, 1940) have remarked, there is little reliable data on which the commonly quoted standards of normality are based. For example the normal left ventricular thickness is quoted by Saphir (1937) as from 8 to 10 millimetres, by Boyd (1945) as from 10 to 15 millimetres and by White (1947) as from 8 to 12 millimetres. White states, nevertheless, that in a series of 100 normal cases the average figure found was 14 millimetres, which exceeded the maximum normal value he quoted. Shennan (1935) drew attention to the importance of the site of measurement when he defined

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TABLE I
Sex and Age Distribution

Sex	Number of Cases	Age (Years)						
		20 to 29	30 to 39	40 to 49	50 to 59	60 to 69	70 to 79	80 to 89
Male	65	2	4	5	10	17	19	8
Female ..	37	—	2	4	6	12	10	3
Total ..	102	2	6	9	16	29	29	11

normal left ventricular thickness as 10 to 15 millimetres at the base of the anterior papillary muscle and 3·3 to 5 millimetres at the apex. Discrepancies then will be greatly multiplied if a fixed site for measurement is not defined.

This investigation was undertaken to correlate the weight of the isolated left ventricle with the more commonly measured dimensions—namely, left ventricular thickness and total heart weight. Total ventricular weight was also included in the study, as were the longitudinal sectional areas of the free wall of the left ventricle. The latter was designed primarily for subsequent

TABLE II
Cause of Death in 102 Cases

Sex	Cause of Death			
	Cardiac	Other Vascular Causes	Surgical	Other Causes
Male ..	26	10	3	26
Female ..	10	12	5	10
Total	36	22	8	36

electrocardiographic study; but in so far as it might be expected to bear a closer relationship to the ventricular mass than would thickness measurements, the findings have been included herein.

Available normal data for the weight of the isolated ventricles is relatively scanty, and this was consolidated together with figures obtained from the normal hearts in this series.

As a corollary to the investigation a somewhat cursory survey was undertaken of the effect of short-term formalin fixation on heart weight. Hermann (1925) found that such fixation of the dog heart resulted in an initial weight gain over the first two to four days, followed by a slowly progressive loss. It was therefore necessary to assess this factor.

MATERIAL

A total of 102 adult hearts was studied. The nature of investigations of this type does not permit random sampling of material. All patients are dead, and in this series an electrocardiogram a relatively short time before death was a prerequisite for the main study. There was a considerable bias to the elderly and to those suffering from cardiac disorders in particular and from vascular disease in general. This unavoidable selection was exaggerated in the first 51 cases because the patients comprised largely those in whom left ventricular hypertrophy was anticipated and in whom primary right ventricular hypertrophy was absent.

In Tables I and II are summarized the age and sex distribution of the series and the ultimate cause of death.

METHODS

Pathological

The hearts were removed at post-mortem examination, flushed thoroughly with water and suspended in 10% formol-saline solution. Dissection was carried out as a rule between the fifth and tenth days with extremes of two and eighteen days. The mean fixation time was 6·1 days (standard deviation=3·37).

Serial weighing during fixation at intervals of one to three days for periods of seven to eighteen days was carried out in 11 cases. No consistent or significant pattern of weight change was found. After eight days' fixation the maximal changes, expressed as variation from original total heart weight, did not exceed +0·5% and −1·2% and averaged a loss of less than 0·2%. After nine to eighteen days the maximal weight variation was ±1·2% and the mean a loss of 1%.

Insufficient numbers are available for detailed analysis of the apparent tendency towards slight weight loss after eight days' fixation. It might be anticipated that if weight loss did occur with the human heart, the cycle described by Hermann for the smaller canine heart would be

lengthened. For the purposes of this study, having regard to possible variations in drainage of the fixing fluid when the hearts were removed for weighing, it was concluded that this aspect was of no significance, and it was not investigated further.

At the completion of fixation the hearts were drained of formalin and extracardiac tissue was removed. The *venæ cavæ* and pulmonary veins were amputated at the atria, and the aorta and pulmonary artery immediately above the respective valve rings. Total heart weight was recorded.

The atria were then removed at the atrio-ventricular groove and immediately above the atrioventricular valve rings. Superficial coronary vessels, pericardial fat and pericardium were dissected from the ventricles, and the coronary arteries were opened and examined. Initial incisions were made into both ventricles to remove any adherent mural thrombi, and the total ventricular weight was then recorded. In the second half of the series (51 cases) the free wall of the right ventricle was removed from the septum by the methods of Fulton and his colleagues (Fulton, Hutchinson and Morgan-Jones, 1952). The weights so recorded therefore are those of the free wall of the right ventricle and of the left ventricle *plus* septum in 51 cases and of total ventricular weight in all 102 cases.

Three longitudinal cuts were then made in the left ventricle, from mitral orifice to apex, at right angles to the surface in the anterior, posterior and lateral walls respectively. Papillary muscles were avoided as far as possible, but where they were involved they were amputated flush with the endocardium, as also were large trabeculæ. The thickness of the ventricular walls was measured at three levels in each section. The levels were defined as the maximal thickness, excluding trabeculæ, in the basal and apical quarters and in the middle half. Where difference existed, the average for the two opposing surfaces was taken as the thickness at that site. The mean figures for each, and for all three, longitudinal sections were then recorded.

The surface areas of the three sections were then also determined by fixing them with pins to squared paper, undue tension being avoided.

RESULTS

Two aspects of this subject have been considered: in particular the accuracy with which left ventricular weight can be determined indirectly; less specifically how reliable a distinction may be made between normality and hypertrophy. The latter demands a definition both of the upper limit of normality of left ventricular weight and of the criteria by which its prediction is proposed. In some instances these standards have been extensively studied by others. Table III shows the findings from 16 normal hearts included in this series. Normality was defined as the lack of a clinical history of hypertensive, renal or cardiac disease and the macroscopic absence of coronary, myocardial or valvular disease at autopsy. Since all patients died from natural causes, the sample is not a random representation of normal, healthy individuals.

Normal Ventricular Weight

Because of the paucity of available figures relating to normal ventricular weight, the

TABLE III

Findings in 16 Normal Cases

Case Number	Sex	Age (Years)	Weight in Grammes			Mean Left Ventricular Sectional Area (1/100 Square Inches)	Left Ventricular Thickness		Cause of Death
			Heart	Total Ventricular	Left Ventricle *plus* Septum		Mean	Mid-Lateral	
1	M.	71	220	150	—	150	12·0	12·0	Hepatic cirrhosis
11	F.	69	290	170	—	177	13·5	15·0	Cerebral thrombosis
48	F.	35	177	143	—	177	13·5	16·5	Ulcerative colitis
57	M.	75	250	178	120	186	11·5	13·0	Bronchial carcinoma
58	F.	63	327	183	123	171	11 5	12·0	Pulmonary embolism (post-operative)
59	M.	63	315	213	162	199	13·0	16·0	Hepatic cirrhosis
60	M.	83	350	185	130	162	14·5	14·0	Gastric hæmorrhage
69	M.	84	410	245	173	250	16·0	17·0	Pneumonia
77	M.	21	283	224	165	230	15·0	18·0	Spinal tumour.
81	F.	35	255	192	149	190	15·0	18·0	Appendicitis
82	M.	32	398	234	169	213	14·0	15·0	Leuchæmia
86	F.	41	182	136	97	112	7·5	9·5	Pulmonary tuberculosis
88	M.	53	258	160	119	197	13·0	12·5	Hepatic cirrhosis
91	M.	84	385	237	183	194	14·5	17·5	Œsophageal carcinoma
96	F.	75	309	203	144	—	—	—	Intestinal obstruction
99	F.	66	297	198	146	218	16·0	17·0	Peritonitis

TABLE IV

Ventricular Weights (Adult)

Authority	Number of Cases	Weight in Grammes					
		Total Heart		Left Ventricle *plus* Septum		Total Ventricular	
		Range	Mean	Range	Mean	Range	Mean
Muller (1883)[1] ..	154 male 137 female	— —	— —	— —	— —	— —	194·8 179·3
Lewis (1914)	10 accident 11 cancer	198–392 240–379	287 304	— —	— —	104·5–221 104 –207	159 149
Hermann and Wilson (1922)[2]	13	—	254	68–155	110	198 –218	159
Levine and Carr (1933)[3]	3 normal 5 wasting disease	218–234 172–267	224 224	— —	— —	102 –165 95 –155	129 138
Fulton *et alii* (1952) ..	43	—	—	65–184	125	88 –235	171
This series	16	177–410	294	97–183 (13 cases only)	145	136 –245	190·5

[1] Muller (1883), quoted by Lewis (1914).
[2] Three cases in subjects under 19 years omitted. Corrected for weight loss.
[3] Non-European cases omitted.

present findings have been summarized with those of other workers in Table IV.

It is evident that the only sample of significant size is that of Muller (1883). No copy of the original manuscript was available, and the information herein was obtained from Lewis

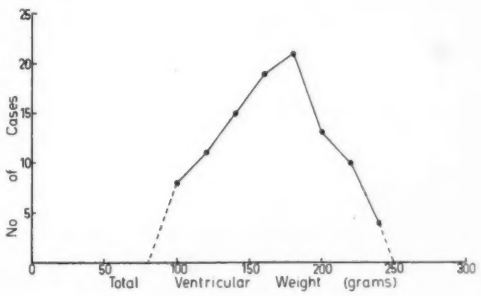

FIGURE I

(1914), who claimed that the series was unselected and may have included some hypertrophied hearts. A considerable variation is seen in the mean values found by all the various authors for total ventricular weight, and this must be anticipated from the small numbers involved. Insufficient details is available from the largest series (Muller, 1883) to warrant analysis, but an aggregate of 101 cases is obtainable from the other figures. From these a distribution curve for normal total ventricular weight has been constructed (Figure I).

From these data the probability of a normal ventricle exceeding 250 grammes in weight can be calculated.

Mean total ventricular weight (grammes) .. .. =166·337
Mean square deviation .. =139·4455
Standard deviation .. .. = 36·852
250 grammes=mean (166·337)+2·27× standard deviation. Alternatively $P<0·01$.

It may therefore be justifiable to accept 250 grammes as the upper limit of normal for all practical purposes; but if a higher degree of significance is demanded (*e.g.* mean weight+3× standard deviation), a normal limit of 277 grammes is defined.

The Determination of Left Ventricular Weight

1. *By Total Ventricular Weight.*—The relation between total ventricular weight and left ventricular weight (as defined by left ventricular *plus* septal weight) in the 102 cases included in the present study is shown in Figure II.

The close correlation could be expressed as a linear regression equation or correlation coefficient.

However, the material involved is not sufficiently homogeneous to ensure that such exact statistical deductions would be generally applicable, and less rigid inferences are preferable.

The conclusion of Fulton, Hutchinson and Morgan Jones (1952) is thus confirmed—namely,

that in the absence of isolated right ventricular hypertrophy, total ventricular weight is a reliable quantitative measure of left ventricular weight. As might be anticipated, it is particularly true of the smaller hearts in which

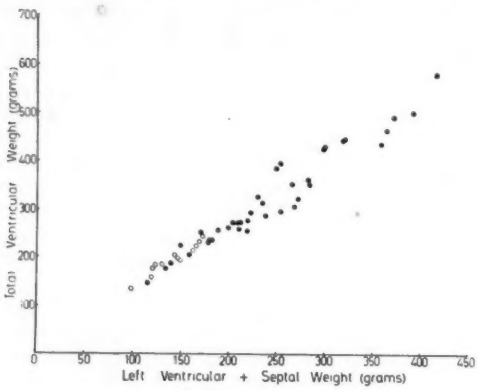

FIGURE II

variable, secondary, degrees of pathological right ventricular hypertrophy (Thompson and White, 1936) are minimal or absent.

Fulton and his associates have suggested that the maximal normal limit of left ventricular plus septal weight is 185 grammes. Where this was exceeded, the total ventricular weight was always

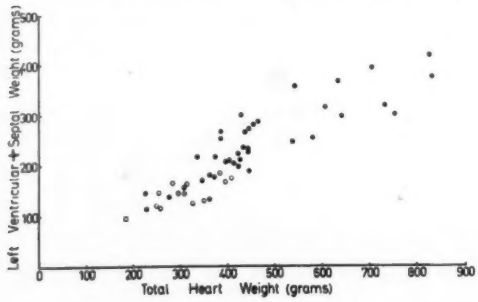

FIGURE III

more than 250 grammes. Conversely, where total ventricular weight exceeded 250 grammes, the figure was never less than 189 grammes, and hypertrophy was therefore present. It is justifiable to conclude that, at least in the absence of primary right ventricular hypertrophy, a total ventricular weight exceeding 250 grammes indicates hypertrophy of the left ventricle. Conversely, in the presence of left ventricular hypertrophy, total ventricular weight will exceed 250 grammes for all practical purposes.

2. *By Total Heart Weight.*—Figures III and IV demonstrate the accuracy with which, in this series, total heart weight could be used to predict, respectively, left ventricular *plus* septal weight as measured directly; and as defined by total ventricular weight.

As might be anticipated there is little material difference in the scatter seen in the two diagrams, Figures III and IV, and, since more cases (98 as opposed to 51) are involved, only the relation of total heart weight to total ventricular weight will be discussed in detail.

Figure IV discloses a relatively close linear correlation between the two measurements in the majority of cases, but a considerable scatter is apparent even with hearts of normal weight, whether healthy or diseased.

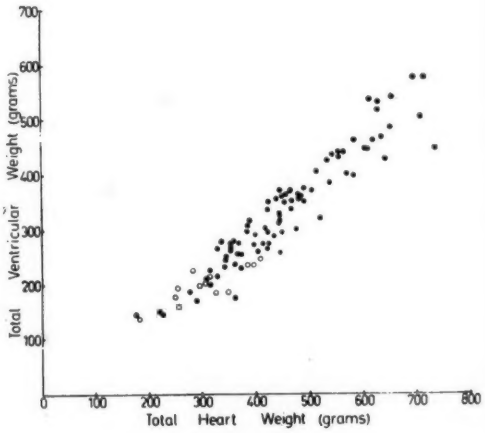

FIGURE IV

Expressed crudely it can be said that non-ventricular structures represented from 19% to 47% of the total heart weight in normal and 17% to 57% in abnormal cases, but that the extremes of the range of variation from the mean were not commonly encountered.

Normal heart weight has been studied by many authors, but the series of Smith (1928) of 1000 cases and of Zeek (1942) of 933 cases are the most extensive and most carefully selected. Nevertheless there is an appreciable difference in their recorded figures:

Smith: Adult males: 155–400 grammes—mean 294 grammes. Adult females: 110–365 grammes—mean 250 grammes.

Zeek: Adult males: mean 319 grammes—standard deviation 40·9 grammes. Adult females: mean 262 grammes—standard deviation 30·2 grammes.

The total heart weight in the 16 normal cases in the present series (Table VI: male 321·7 grammes, female 262·5 grammes) approximates that of Zeek.

It is evident that the chance of a normal heart exceeding 400 grammes in weight is slight though appreciable. If this figure be taken as the upper limit of normality, then left ventricular hypertrophy (total ventricular

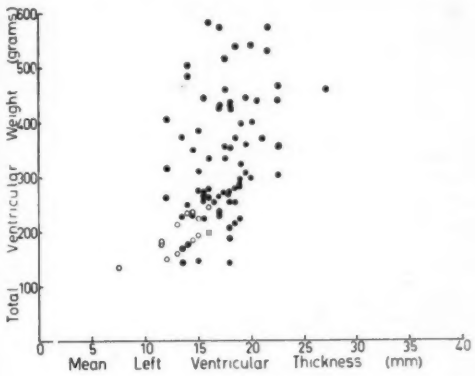

FIGURE V

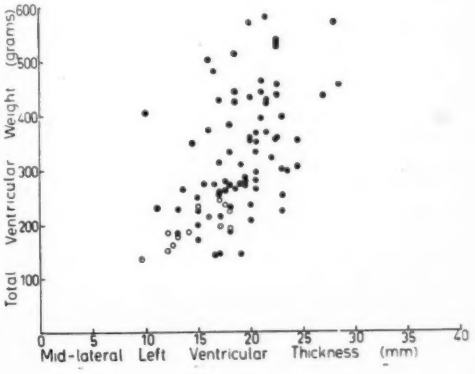

FIGURE VI

weight over 250 grammes), as denoted by a total heart weight exceeding 400 grammes, would have been predicted correctly in 55 of 67 cases (82%) in which it was present and incorrectly only once in the 31 cases in which it was absent. However, its presence would have been overlooked in 12 cases, and this error would not be eliminated unless a total heart weight of 320 grammes were elected as the diagnostic level. Such a choice would then introduce 13 false positives, a 16% error in prediction.

It may be concluded then that only where total heart weight exceeds 400 grammes is left ventricular hypertrophy probably present, and that where it is less than 320 grammes is such hypertrophy unlikely. No valid deduction can be drawn in the range of 320–400 grammes. From the quantitative aspect a parallel exists between total heart weight and left ventricular weight, but, at any selected total heart weight, total ventricular weight cannot be predicted with a precision of greater than ±10% to 20%. This error applies whether or not normal ventricular weight is defined as an absolute figure or in terms of body stature.

3. *By Left Ventricular Thickness.*—Cases in which material myocardial destruction had resulted from infarction were omitted, and a total of 89 hearts remained for study. The mean left ventricular thickness obtained from nine sites, and the single mid-lateral thickness measurement, are related to total ventricular weight in Figures V and VI respectively. (In these and subsequent figures normal hearts are shown by an open circle and others by a solid circle.)

In neither case does more than gross quantitative correlation exist, and it is apparent without further analysis that left ventricular thickness is or no real value in the prediction of left ventricular weight. When the mean thickness exceeded 19 millimetres, hypertrophy was always present; and when it was below 11 millimetres, hypertrophy was absent. Above 17 millimetres 38 of 47 ventricles were hypertrophied, and below this 25 of 42 were not. A single thickness measurement taken in the middle half of the lateral wall of the left ventricle was of even less value. Unless it exceeded 23 millimetres, left ventricular hypertrophy was not invariably present.

An average left ventricular thickness of 16 millimetres and a lateral wall thickness of 18 millimetres were sometimes found in normal hearts. It is considered that figures often quoted as normal are too low. These results (Table III) support the figures of White (1947), who found the average single thickness measurement to be 14 millimetres in the mid-lateral wall of the left ventricle of 100 normal hearts. The mean value for 16 normal hearts in this series was 13·5 millimetres.

4. *By Left Ventricular Longitudinal Sectional Areas.*—The relation of the mean and anterior wall sectional areas to total ventricular weight is seen in Figures VII and VIII.

The mean sectional dimension offers better correlation than the single figure, but neither affords a more accurate prediction of total

(left) ventricular wieght than does total heart weight. The value of the mean figure is of comparable value in that no normal sectional area exceeded 250 (1/100 square inch units). When this figure was surpassed only two cases failed to show ventricular hypertrophy. However, seven false negative predictions were seen,

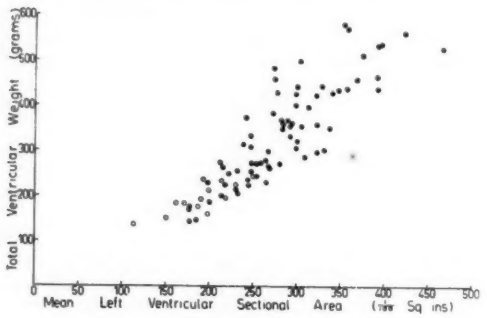

FIGURE VII

which are not significantly different from the 12 found within the normal range of total heart weight. Since total heart weight can be determined very simply it is obviously to be preferred.

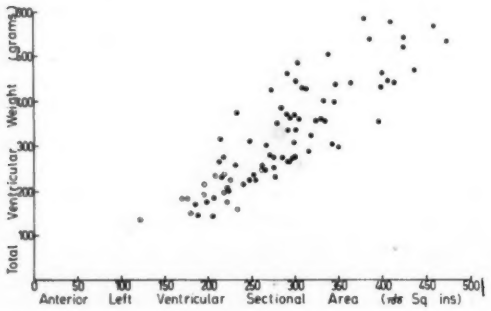

FIGURE VIII

DISCUSSION AND CONCLUSIONS

Formalin Fixation.—Although prolonged fixation of the human heart in formalin may cause progressive weight loss, this is not significant within the first eighteen days.

Normal Ventricular Weight.—Statistical analysis of the findings in 101 normal hearts collected from the present series and the literature confirms the unsubstantiated opinion of Fulton, Hutchinson and Morgan Jones (1952) that for practical purposes 250 grammes may be taken as the upper limit of normal total ventricular weight. However, for a high degree of statistical significance ($P<0{\cdot}001$) a normal limit must be defined as 277 grammes.

Left Ventricular Hypertrophy.—It is a common practice not only for pathologists at routine autopsy but also for those embarking on specialized investigations to assess left ventricular hypertrophy from total heart weight and sometimes from left ventricular thickness. Although the practice has been occasionally decried, no specific data have been published previously to assess the reliability of the methods.

Total Heart Weight.—Analysis of 102 cases revealed that the percentage of total heart weight represented by non-ventricular tissues varied from 19% to 47% in 16 normal cases and from 17% to 57% in hypertrophied hearts. This widely variable potential error is of course extreme, but in practice it can only be said that in the absence of primary right ventricular hypertrophy a total heart weight exceeding 400 grammes almost always indicates left ventricular hypertrophy. The converse does not hold, as in 18% of the present series left ventricular hypertrophy was present, with a total heart weight of less than 400 grammes. When total heart weight is less than 320 grammes hypertrophy of the left ventricle is improbable. Alternatively, total heart weight cannot predict total ventricular weight with an accuracy exceeding some $\pm 10\%$ to 20%. However, it should be noted that in all cases in which total ventricular weight exceeded 300 grammes, or 20% above the maximum normal weight, total heart weight always exceeded 400 grammes. Therefore, as a simple procedure for routine assessment at autopsy, total heart weight is a useful guide to the presence or absence of marked left ventricular hypertrophy. For the purpose of accurate study, however, actual ventricular weight must be determined directly.

Left Ventricular Thickness.—Left ventricular thickness is valueless as a method of estimation of left ventricular weight irrespective of whether it be measured at one or many sites. Although too few normal cases were examined for the figures to be of any real significance, they support the view that many reputed standards of normality are, in fact, too low.

Longitudinal Sectional Areas.—Longitudinal sectional area measurement provides no more reliable guide to left ventricular weight than does the far more simple procedure of weighing the intact heart.

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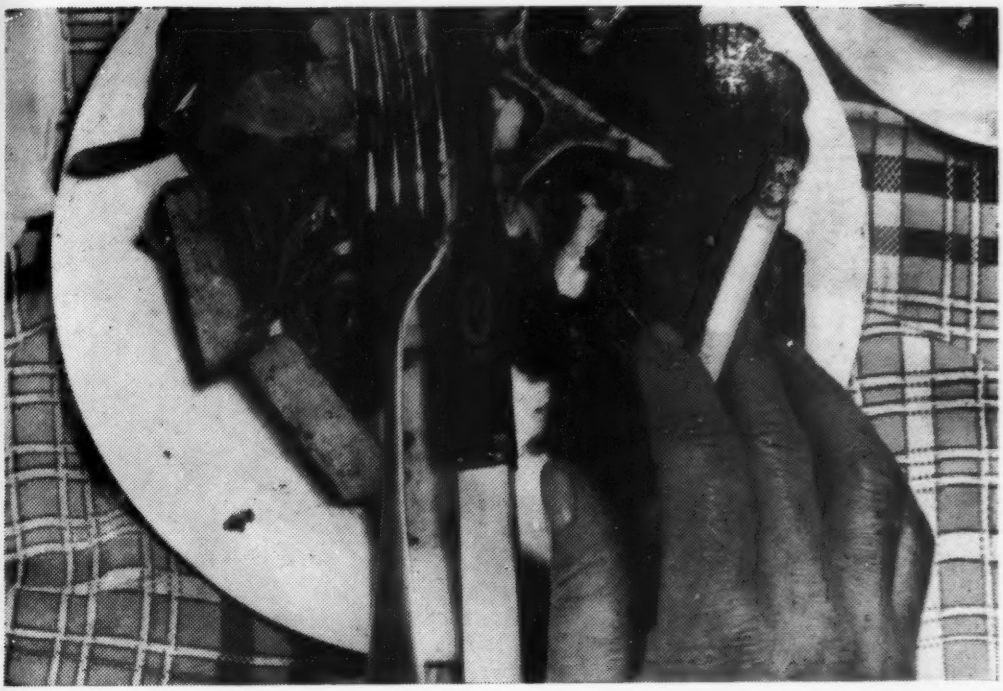

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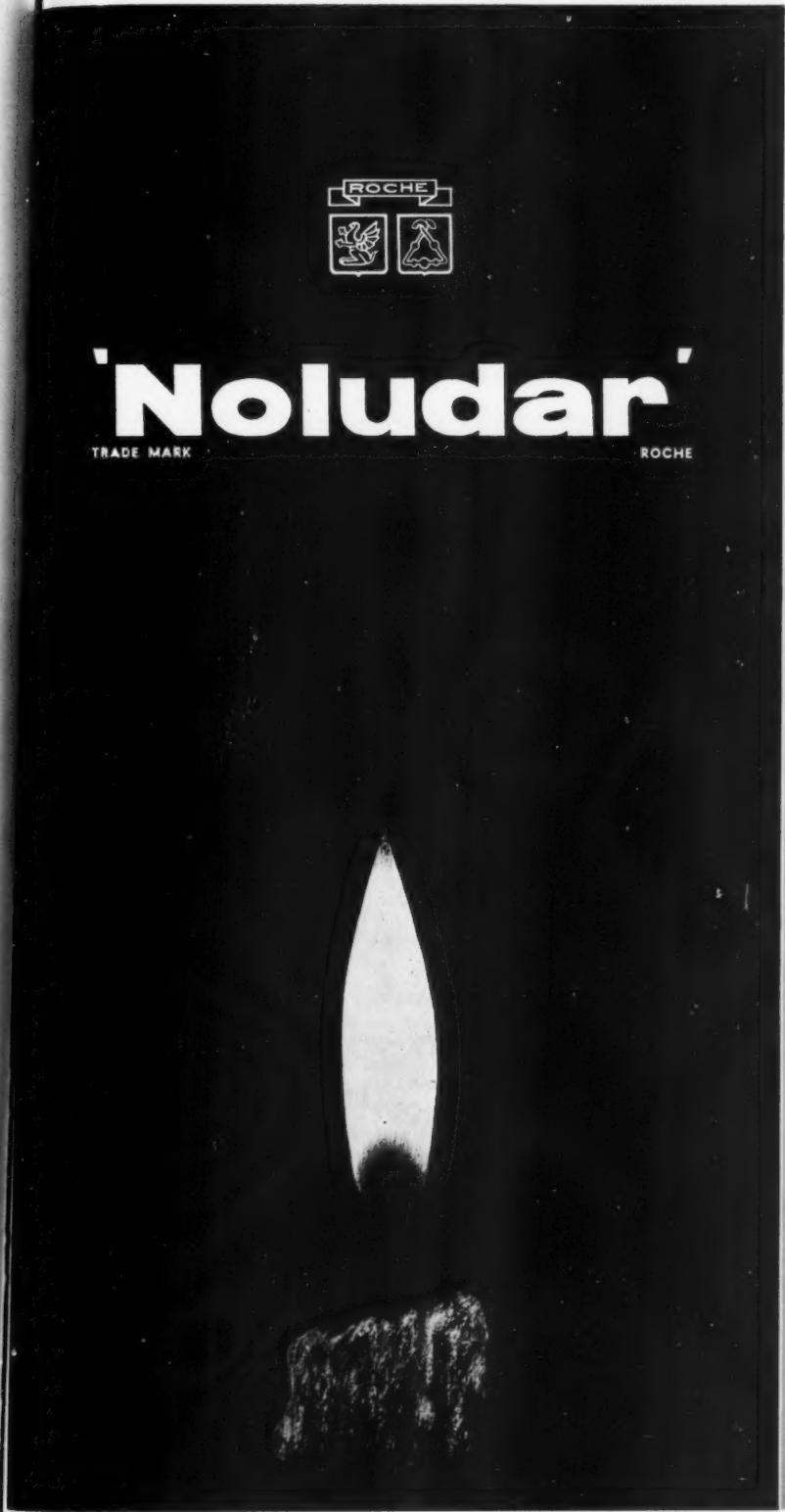

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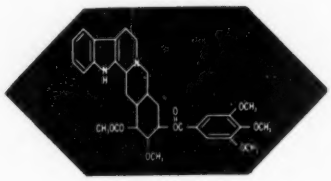

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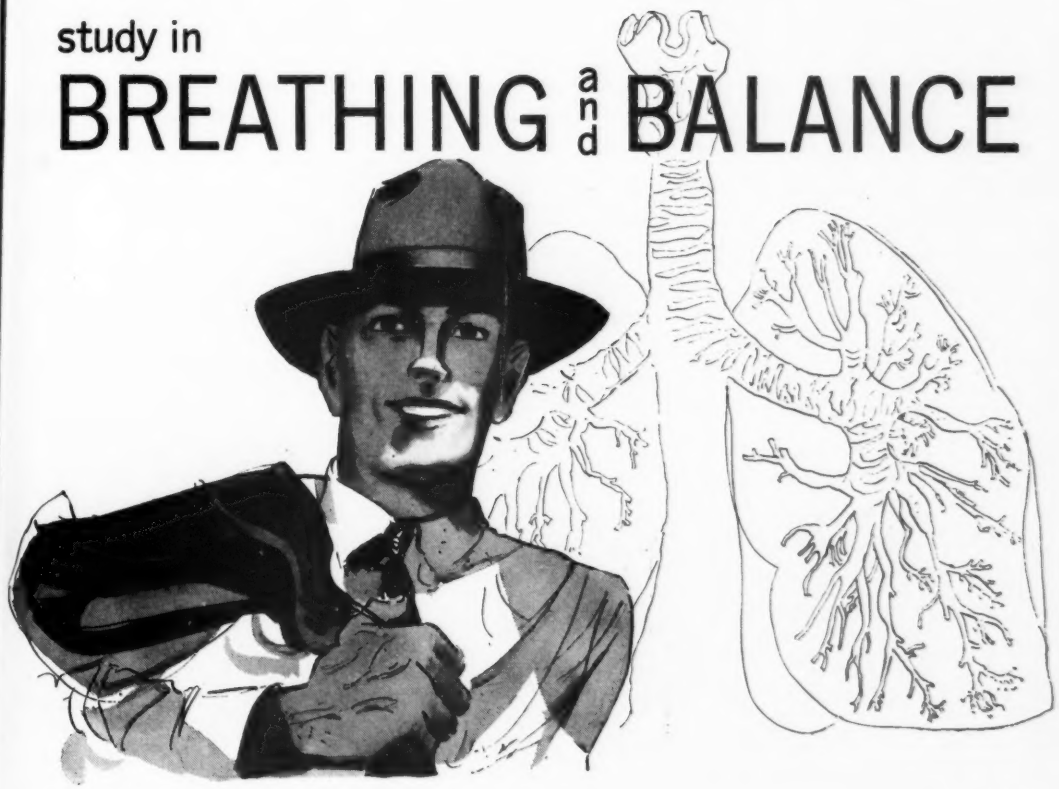

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